"OLD-GOLD" FLOWER COLOR, THE SECOND CASE OF INDEPENDENT INHERITANCE IN OENOTHERA[1]*

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INTRODUCTION

The evening primroses display, as sharply as do any other plants or any animals, a mosaic-like combination of alternatively inherited characteristics, such as provide in most other organisms the clearest examples of typical Mendelian inheritance. A list of the more striking of these alternative characters includes: (a) tall *versus* dwarf stems; (b) red *versus* green stems; (c) red-spotted *versus* unspotted green leaves; (d) flat leaves *versus* revolute leaves; (e) narrow petals *versus* broad petals; (f) yellow petals

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versus sulfur petals; (g) red hypanthium *versus* green hypanthium; (h) red or pink bud-cones *versus* green bud-cones; (i) long styles *versus* short styles. The first-mentioned member of each of these pairs is the dominant. In addition to these, there appear to be at least four recessive lethals paired with four corresponding dominant "vital" factors. Of all these factors and some others which are known but not here enumerated, only the short-styled form of *Oenothera brevistylis* has been found, heretofore, to be distributed in inheritance independently of the others.

The relation between the long styles of *Oenothera Lamarckiana* and the short styles of *brevistylis* may be considered a classic example of Mendelian inheritance, as this pair of contrasting characters was included by DE VRIES (1900) in the list of 11 examples cited in his original statement of the "law of disjunction of hybrids,"—one of the three announcements of the three independent rediscoveries of the Mendelian laws.

Were no lethal factors involved, each of the other character pairs mentioned above should likewise yield typical Mendelian phenomena when handled singly in the production of monohybrids; that is, allowing for errors of random sampling and deviations due to selective viability, etc., they would give uniform F_1 families of the dominant type; would split in F_2 families, following selfing of the F_1, into 3 dominants to 1 recessive; on backcrossing to the recessive, they would give 1 : 1 ratios of dominants to recessives; and reciprocal crosses would give identical results.

The wide-spread occurrence of lethal factors has so modified this program that the Oenotheras have long seemed an outstanding exception to the general applicability of the Mendelian scheme of heredity.

I have shown in previous papers (SHULL 1921a, b, 1923a, b) that this disturbing effect of the lethals is due to the fact that all of the above-mentioned factors, except the pair for long and short styles, are associated together in a single linkage group (group I) in which the lethals likewise occur. I have assumed that the linkage of these factors is the result of their inclusion in a single pair of chromosomes, but recent cytological studies of CLELAND (1922, 1923, 1924, 1925, 1926) indicate the existence of a possibly alternative mechanism for the production of linkage in the Oenotheras, and it seems best for the present to admit the possibility that linkage may result from chromosomal cohesions (synzeuxis), though I still think that the available evidence is more favorable to the view that linkage in the Oenotheras, as in other organisms, is based on the inclusion of the linked genes within a single chromosome pair (monozeuxis).

This new fundamental problem as to the physical basis of linkage in the Oenotheras adds to the interest and importance of further genetical

studies in this group of plants, and gives special significance to any factors which may be found to lie outside the big linkage group I with which nearly all studies have heretofore been concerned.

The factor for old-gold flower color (mut. *vetaurea*) has been the first, after *brevistylis*, to fulfill this condition. One of the first families in which it appeared (2047) showed plants of the *Lamarckiana* type segregating 40 yellow : 13 old-gold. In 1922 and 1923 self-fertilized *Lamarckiana* plants in the line of direct descent from the family in which the original mutation occurred, yielded typical 3 : 1 ratios of yellow and *vetaurea*-flowered *Lamarckiana*, namely, (No. 2123) 35 Y : 8 V, and (No. 2220) 22 Y : 7 V. These 3 : 1 segregations in typical *Lamarckiana* families, have been paralleled, heretofore, only by the segregation to the short style of *brevistylis*, and show that the *vetaurea* factor is not in the same linkage group as the *Lamarckiana* lethals,—therefore, not in group I.

The fact that *brevistylis* was the only previously known character which behaved in this way, made it seem desirable to test very completely the genetical relations of the old-gold mutation. For this purpose it has been a very fortunate circumstance that the old-gold factor has no noticeable injurious effects on the reproductive processes. It is thus greatly superior to *brevistylis* for such a study, since *brevistylis*, because of the deformed and disadvantageously placed stigmas, can be used as a seed parent only with the greatest difficulty, though it produces abundant pollen, and is therefore a very satisfactory pollen parent.

The present paper gives a complete account of the breeding results with the old-gold factor, with the exception of a few recent crosses made in an attack on certain problems which are not yet ready for report.

ORIGIN OF THE OLD-GOLD MUTATION

As described in a previous paper (SHULL 1921b) the old-gold mutation (mut. *vetaurea*) manifested itself in 1921 in two families (2047 and 2052), both of which had the same individual grandparents 188 (12)×187 (56), the parents having been two individuals, (30) and (54), in family 198. A portion of the network of cross-breedings from which these two families were derived is shown in figure 1. With exception of the year 1914, this network of descent has been continued in the same manner, with annual generations, since 1905, when it was begun with ten rosettes of *Oenothera Lamarckiana* collected at my request by Professor HUGO DE VRIES, in the field at Hilversum, Holland, from which he had secured the foundational material for his own cultures almost 20 years previously. Every plant used in this network has been a typical *Lamarckiana*, and every

family in this series of cultures has had the usual composition of *Lamarckiana* families, nearly all of the offspring in each progeny being typical *Lamarckiana*, but usually associated with a small percentage of mutant types, the majority of which have been recognizable as 15-chromosome forms. Only mut. *funifolia* (SHULL 1921b), discovered in 1918, and mut. *vetaurea*, the subject of the present paper, have been demonstrably pro-

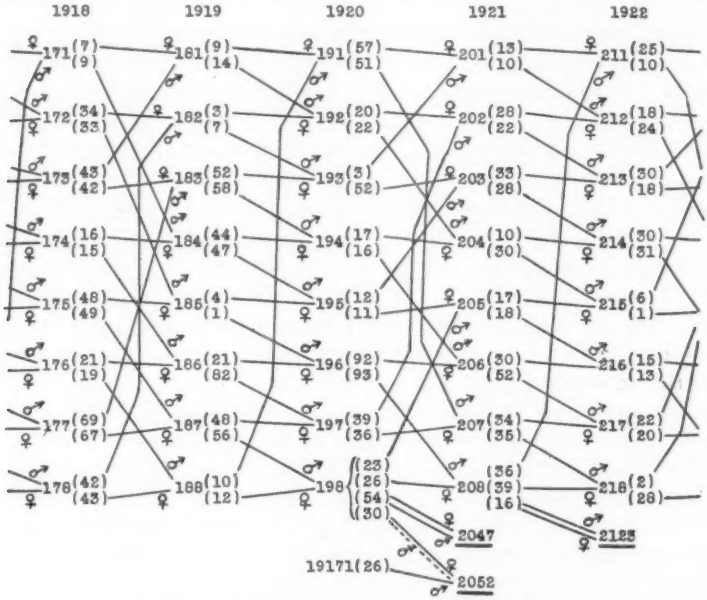


FIGURE 1.—Five generations of the network of descent in which "old-gold" originated as a gene-mutation. The three-figure numbers are family numbers, and the numbers in parenthesis are individual numbers. The year in which each generation was grown is indicated at the top of the corresponding column. Every individual represented was a typical *Lamarckiana* except 198(54) which was a green-coned mutant. Only in families 198 and 208 was the presence of old-gold as a hidden recessive type demonstrated. The pedigree numbers of families in which old-gold plants made their appearance *de novo* are underscored. The dotted line connecting 198(30) with 2052 represents an accidental self-fertilization, which alone made it possible for a *vetaurea* plant to appear in family 2052.

duced as the result of gene mutations, and I believe that these are the only new characters in Oenothera which have been certainly ascertained to have been the result of gene mutations, as distinguished from the process of unmasking recessive factors which have been kept hidden because of their linkage with lethal factors.

OLD-GOLD BREEDS TRUE

The first question which presents itself, on the discovery of a new characteristic in any organism, is whether it will breed true. Experience teaches that many such new forms are due to some accident of development that produces no effect whatever on the offspring; that others are apparently the result of complex combinations of genes which by chance only rarely recombine in the grouping necessary to produce again the characteristic in question; that some produce progenies which return in part to the parental type; and that still others segregate into one or more subordinate types. In table 1 are presented the results of 21 self-fertilizations of the old-gold type (mut. *vetaurea*), and of 14 crosses between two plants of the same type. The 35 families contained a total of 1772 old-gold, and none of any other type.

As will be seen in a later section, however, old-gold may segregate a subordinate type,—but only when it is not derived from a pure yellow (or old-gold) parentage.

OLD-GOLD RECESSIVE TO YELLOW

That the old-gold factor is recessive to yellow was indicated by the splitting of the first families observed, in 3 : 1 ratios of yellow to old-gold. The numerous crosses which have been made between forms having yellow flowers and forms with old-gold flowers, have always given only yellow flowers, except, of course, when the yellow-flowered form had been rendered heterozygous by a previous cross. Table 2 shows the results of 30 such crosses, the total F_1 yield of which was 1961 yellow-flowered individuals and none of any other color.

The most important series of crosses included in this table are those which involved as one of their parents an individual in families 201 to 208, for these are the families belonging to the network of descent shown in figure 1, in which the old-gold mutation occurred. The yellow-flowered parent in each of these nine families stood in a relation of cousinship of greater or less degree of closeness to the original mut. *vetaurea*. The fact that every one of these crosses yielded only yellow-flowered offspring in the F_1 demonstrates that the *vetaurea* factor was not present in the strain, hidden, as in some other Oenothera mutations,—for example *nanella* and *pervirens*,—by the presence of lethal factors linked with them. This renders it certain that the *vetaurea* factor originated as a gene mutation affecting one of the two gametes whose union gave rise to one of the parents of family 198, in which family, as we have seen, several individuals were demonstrably heterozygous for the *vetaurea* factor.

TABLE 1

Families which illustrate the true-breeding of mut. vetaurea.

PEDIGREE NUMBERS		SEEDS SOWN	PLANTS SECURED	PERCENTAGE GERMINATION	NUMBER FLOWERED, ALL VETAUREA
Parents	Family				
2052(10)	21108	300	63	21.00	45
2047(40)	21109	20	1	5.00	1
2047(92)	21113	184	127	69.02	105
2047(39) × 2047(92)	21114	184	78	42.40	64
21108(19)	22147	300	79	26.33	3
21113(5)	22148	300	168	56.00	38
21159(63)	22216	300	123	41.00	36
21159(113)	22217	300	140	46.67	85
21108(19)	2389	300	66	22.00	44
2220(40)	2390	300	28	9.33	25
22148(69)	2391	261	119	45.59	55
2220(46) × 22162(7)	2393	300	124	41.33	112
22167(41)	2398	300	183	61.00	86
22216(77)	23136	300	34	11.33	34
23135(5)	2486	300	172	57.33	107
23137(92)	24101	300	50	16.67	43
23137(92) × 23137(80)	24102	300	212	70.67	143
23137(46) × 23137(92)	24103	26	10	38.46	10
23137(91) × 23137(92)	24104	300	70	23.33	54
2389(44) × 23137(92)	24105	300	94	31.33	50
23137(92) × 23144(30)	24112	300	85	28.33	61
23137(105)	24114	147	69	46.94	49
23137(46) × 23137(105)	24115	24	7	29.17	5
23137(91) × 23137(105)	24116	300	116	38.67	57
23137(46) × 23137(80)	24119	35	7	20.00	3
23137(91) × 23137(80)	24120	300	230	76.67	95
23137(91) × 23137(63)	24124	300	217	72.33	133
23137(91) × 23137(76)	24125	300	138	46.00	98
23137(91)	24126	300	56	18.67	31
23137(18)	24127	76	29	38.16	7
23137(46)	24128	154	53	34.42	15
23142(2)	24148	300	78	26.00	32
23142(2) × 23144(30)	24149	245	37	15.10	30
2390(5)	24150	300	54	18.00	5
2391(43)	24151	25	12	48.00	11
Total					1772

TABLE 2

Families which show the recessiveness of vetaurea in the F_1 of crosses with homozygous yellow.

PEDIGREE NUMBERS			SEEDS SOWN	NUMBER OF PLANTS SECURED	PERCENTAGE GERMINATION	NUMBER FLOWERED, ALL YELLOW
Parents ♀	♂	Family				
2047(39)	2047(42)	21111	300	154	51.33	126
2047(40)	2046(159)	21115	134	42	31.34	40
2046(159)	2047(40)	21117	300	138	46.00	111
2047(39)	20195(1)	21118	168	64	38.10	60
2047(39)	20247(47)	21119	20	3	15.00	3
2047(39)	20183(36)	21123	323	115	35.60	113
2047(39)	20202(17)	21126	85	22	25.88	20
2047(39)	201(10)	21128	78	21	26.92	16
201(13)	2047(39)	21129	139	14	10.07	11
202(28)	2047(39)	21130	175	21	12.00	20
203(33)	2047(39)	21131	269	46	17.10	38
205(17)	2047(39)	21133	141	15	10.64	11
206(30)	2047(39)	21134	300	104	34.67	90
207(34)	2047(39)	21135	300	121	40.33	85
208(39)	2047(39)	21136	154	21	13.64	17
2047(39)	208(36)	21137	300	97	32.33	84
2047(39)	2031(13)	21138	161	97	60.25	94
2031(13)	2047(39)	21139	300	134	44.67	105
22148(69)	2295(43)	2392	169	103	60.95	90
22218(34)	22162(7)	23139	300	70	23.33	64
237(23)	23137(92)	24106	300	148	49.33	129
23138(44)	23137(92)	24110	300	94	31.33	87
23138(73)	23137(92)	24111	300	106	35.33	95
237(23)	23137(105)	24117	158	61	38.61	19
23138(73)	23137(105)	24118	300	91	30.33	52
237(23)	23137(80)	24121	259	201	77.61	143
23138(73)	23137(80)	24122	300	165	55.00	87
23137(46)	23138(73)	24131	34	3	8.82	3
23138(73)	23137(46)	24132	300	167	55.67	144
23137(46)	23138(73)	24133	49	4	8.16	4
Total						1961

SEGREGATION OF OLD-GOLD FROM SELF-FERTILIZED HETEROZYGOTES

The F_2 families derived from crosses between homozygous yellow and old-gold have generally consisted of yellow-flowered and old-gold-flowered plants, in ratios which do not deviate too seriously from the 3 : 1 ratio, when the size of family is taken into account. Twenty-four families of this type are presented in table 3, and it is seen that the totals show 1005 yellow-flowered to 264 old-gold, where 3 : 1 ratios should have given 952 yellow to 317 old-gold. This very material deviation, which is paralleled

in some of the other segregations in which the old-gold factor was concerned, might be taken to indicate that, while there is no noticeable inferiority of plants possessing old-gold flowers as compared with those which have yellow-flowers, there is, nevertheless, a probable differential elimination of the old-gold type. There is a special consideration which must be taken into account, however, which may modify the stress to be laid on the deviations shown by the ratios in table 3 and in several of the other tables. The seasons of 1922 and 1923, especially the latter, were very unfavorable because of long periods of drouth, and as a result these years were marked by a failure of many plants, especially of the *Lamarckiana* type, to come to bloom.

In certain families it has been quite obvious that the old-gold type has been slightly later in coming to bloom, than the yellow-flowered type, and it is not unlikely that this slightly delayed development has been effective in decreasing the number of old-gold relatively to the yellow, when conditions were such that only a small portion of the plants secured were induced to bloom. A rather striking illustration of this is seen in families 22155 and 22160, produced by the selfing of two heterozygous sibs in family 21117. In the former, only ten plants bloomed, out of 151, and these were all yellow-flowered, while in 22160 in which 77 plants out of 134 bloomed, there were 61 yellow-flowered to 16 old-gold.

THE YELLOW SEGREGATES FROM SELF-FERTILIZED HETEROZYGOTES

The occurrence of 3 : 1 ratios following self-fertilization of *Oenothera Lamarckiana* having been previously known only in crosses of *Lamarckiana*×*brevistylis*, it seemed important to determine, experimentally, whether the dominant class had the composition which Mendelian theory required, namely, one-third homozygous yellow and two-thirds heterozygous yellow, or whether the 3-to-1 grouping was the result of some other cause or causes than the distribution of a pair of factors in such manner that equal numbers of sperms and equal numbers of eggs carried each member of the pair.

In order to determine this point, the family 2123 (see table 3 and figure 1) was made the subject of an analysis of its dominant type. Of the 35 yellow-flowered plants in this family, 34 were self-fertilized, and the resulting cultures grown in 1923. Unfortunately, there was a seven-weeks drouth in the spring of that year, in the midst of which these plants were transplanted from the greenhouse, where they had received daily irrigation, to the experimental field where the artificial application of water was not feasible. In consequence, while there were very few actual losses

by death of the plants, the development was so long retarded that most of the plants resumed the biennial habit which is normal for the species, making large rosettes but developing no stems. Very few of the rosettes lived over the following winter, and no attempt was made to flower any of them in the next season. The scanty data secured from these families are recorded in table 4. With numbers in each family so small, the results

TABLE 3

Families from self-fertilized heterozygotes, from crosses between homozygous yellow and velaurea. See also table 4.

PEDIGREE NUMBERS		SEEDS SOWN	PLANTS SECURED	PERCENTAGE GERMINATION	PLANTS FLOWERED	
Parent	Family				Yellow	Old-gold
198(54)	2047	164	94	57.32	42	20
208(16)	2123	300	62	20.67	35	8
2047(50)	21110	75	12	16.00	3	3
2052(11)	21158	148	22	14.87	11	3
2052(59)	21159	300	129	43.00	88	25
21115(11)	22150	260	160	61.54	65	9
21117(22)	22155	300	158	52.67	10	0
21117(62)	22160	300	134	44.67	61	16
21118(13)	22162	300	40	13.33	16	5
21118(52)	22163	300	173	57.67	85	14
21119(2)	22167	300	103	34.33	35	5
21120(4)	22170	300	73	24.33	21	11
21123(53)	22188	300	149	49.67	45	14
21126(5)	22208	300	90	30.00	50	16
21130(1)	22209	282	135	47.87	7	4
21130(20)	22210	300	144	48.00	71	15
21130(7)	22211	300	76	25.33	5	0
21139(22)	22212	139	45	32.37	10	3
22166(43)	23144	300	160	53.33	100	24
23137(94)	24129	300	45	15.00	21	8
23137(100)	24130	300	132	44.00	94	22
23139(16)	24144	300	96	32.00	49	10
23139(53)	24146	300	58	19.33	31	7
2392(30)	24152	300	111	37.00	60	22
Total					1015	264
Expected (3:1)					*959.25*	*319.75*

are necessarily indecisive, but they are at any rate not in disagreement with the expected results. The 34 families, if distributed as nearly as possible in a 2 : 1 ratio, would have consisted of 23 splitting and 11 non-splitting families, but actually, segregation was manifested in only 13 families and no segregation in 21. When it is observed, however, that one family had no flowering specimen, 3 had only one each, two had only two

each, and so on, and that 24 families had not more than six individuals each, it will be readily seen that a number of potentially splitting families, due alone to the errors of random sampling, will have failed to exhibit a single individual of the recessive type, and such families, consequently, must be grouped with non-splitting families. Assuming that the distri-

TABLE 4

Tests for the occurrence of homozygotes and heterozygotes in Lamarckiana family 2123, which segregated to 35 yellow and 8 vetaurea. All but one of the yellow-flowered plants were self-fertilized.

PEDIGREE NUMBERS		SEEDS SOWN	PLANTS SECURED	PERCENTAGE GERMINATION	PLANTS BLOOMED	
Parent	Family				Yellow	*Vetaurea*
2123(1)	22111	300	84	28.00	56	9
(2)	22112	150	33	22.00	2	–
(3)	22113	300	60	20.00	7	–
(4)	22114	300	85	28.33	16	–
(5)	22115	300	32	10.67	3	2
(6)	22116	300	27	9.00	4	–
(9)	22119	300	24	8.00	10	1
(10)	22120	300	22	7.33	4	1
(11)	22121	300	84	28.00	18	–
(12)	22122	300	83	27.67	6	–
(13)	22123	300	47	15.67	4	–
(14)	22124	300	72	24.00	8	–
(15)	22125	300	77	25.67	2	1
(16)	22126	300	72	24.00	20	1
(17)	22127	300	90	30.00	1	2
(18)	22128	300	115	38.33	4	–
(19)	22129	300	121	40.33	12	2
(23)	22130	300	86	28.67	4	1
(24)	22131	300	48	16.00	5	–
(25)	22132	300	46	15.33	5	–
(26)	22133	300	56	18.67	1	–
(29)	22134	300	65	21.67	–	–
(30)	22135	300	70	23.33	1	–
(31)	22136	188	21	11.17	6	–
(33)	22137	300	57	19.00	4	–
(34)	22138	300	86	28.67	1	1
(35)	22139	300	58	19.33	10	–
(36)	22140	300	53	17.67	4	–
(37)	22141	300	58	19.33	1	–
(38)	22142	300	95	31.67	1	2
(39)	22143	300	88	29.33	5	–
(40)	22144	300	85	28.33	3	3
(42)	22145	300	73	24.33	10	1
(43)	22146	300	55	18.33	2	1

Summary: { 20 apparently non-splitting families contain 111 yellow;
14 splitting families contain 129 Y : 28 V, or 4.6 : 1.

bution of the potentially segregating and non-segregating families agrees with the expected 2 : 1 ratio, and calculating the probabilities that the recessive type will be actually included within families having the sizes indicated in table 4 as falling between zero and 6 individuals, inclusively, the theoretical grouping of the 24 families to which these specified limits apply gives 14 apparently non-splitting to 10 splitting. The actual grouping of these 24 families, as seen in the table, is 15 apparently non-splitting and 9 splitting, a very close fit to the theoretical expectation on the assumption that the gametes carrying the factor for old-gold and those carrying its dominant allelomorph are equally numerous in both eggs and sperms. The slight deficiency in the number of splitting progenies is not great enough to be significant in itself, but is in apparent harmony with a significant deficiency in the relative number of recessives as compared with dominants, which resulted from the backcrosses discussed in the next section.

YELLOW-FLOWERED HETEROZYGOTES BACKCROSSED TO OLD-GOLD

The backcrosses of the heterozygotes to the recessive, for the purpose of testing the gametic ratios, are given in table 5. The deviations from the

TABLE 5

Heterozygotes from crosses between yellow and vetaurea, backcrossed to the recessive vetaurea.

PEDIGREE NUMBERS			SEEDS SOWN	PLANTS SECURED	PERCENTAGE GERMINATION	PLANTS FLOWERED	
Parents ♀	Parents ♂	Family				Yellow	*Vetaurea*
2047(39)	2047(50)	21112	300	115	38.33	46	44
21115(11)	21108(19)	22151	82	38	46.34	17	5
21117(22)	21108(19)	22157	200	100	50.00	29	39
21117(15)	21108(19)	22158	300	73	24.33	6	5
21117(62)	21108(19)	22159	300	106	35.33	47	28
21118(13)	21108(19)	22164	300	36	12.00	6	5
21119(52)	21108(19)	22165	300	100	33.33	39	15
21119(2)	21108(19)	22168	300	87	29.00	16	9
21123(53)	21108(19)	22189	300	148	49.33	57	44
21126(5)	21108(19)	22200	300	142	47.33	39	27
21139(95)	2223(41)	22213	300	159	53.00	30	2
21139(22)	2223(41)	22214	300	113	37.67	34	20
21139(95)	2223(41)	22215	300	210	70.00	80	42
23139(16)	23144(30)	24145	300	69	23.00	27	22
23139(53)	23144(30)	24147	300	86	28.67	32	31
2392(30)	2391(43)	24153	300	204	68.00	71	58
2392(30)	23144(30)	24154	300	137	45.67	46	38
Total						622	434
Expected (1 : 1)						*528*	*528*

expected 1 : 1 ratio are considerable, but are quite irregular. There is a suggestion in some of the families in this table that there may be present in these particular families a loose linkage of the old-gold factor with a gamete lethal, but this is a subject which will require much more work, and while some experiments are already under way, bearing on this question, they are not yet ready for discussion. It is of special importance to note in this connection, that in most of those families the *vetaurea* grandparents were direct derivatives of *Oenothera Lamarckiana*, in which no gamete lethals have yet been recognized in association with factors of linkage-group I, although the occurrence of abortive pollen and abortive ovules has been repeatedly interpreted as evidence of the occurrence of lethals in this species. At any rate, if the deviations from the 1 : 1 ratio in the families listed in table 5 result from linkage with one or more gamete lethals, it is clear that these lethals are not the same lethals that so strongly dominate the manifestations of factors belonging to group I. The seventeen families listed in table 5 contained a total of 622 yellow-flowered and 434 old-gold,—a deviation from the expected ratio, 528 : 528, too great to refer to errors of sampling, but the nature of the disturbing factors must await the results of further investigation before they can be profitably discussed.

OLD-GOLD COMPLEMENTARY TO SULFUR

Another flower color, also recessive to yellow, has long been known, namely, the *sulfurea* mutation of *Oe. biennis* and of the nearly related species, *Oe. suaveolens*. Sulfur-flowered forms of these two species have been found occasionally in nature in Europe, over a period of time long antedating the beginning of genetical experiments, and they have also arisen by (crossover) mutation in these two species in experimental cultures of STOMPS (1914) and DE VRIES (1918), and once in my own cultures of *Oe. biennis*. The sulfur factor has also been introduced by DAVIS (1916) into *Oe. franciscana* by means of a cross between an *Oe. biennis sulfurea* and *Oe. franciscana*, and I have now, in turn, introduced it into the *Lamarckiana* series of forms. I have made extensive use of the sulfur factor in my genetical experiments, and have reported its close linkage with all of the factors of linkage group I (SHULL 1921a, b, 1923a, b). It has been, until recently, usually associated with one or other of the gamete lethals which control its manifestations in *Oe. biennis* and *Oe. suaveolens*.

One of the first questions which arose, on finding the new recessive flower color, *vetaurea*, was its relationship to the old recessive color, *sulfurea*, and crosses were immediately made to determine this relation-

ship. The ratio of yellow to old-gold in one of the first families containing the latter type, had suggested the possibility that old-gold might be included in the first linkage group, where sulfur had already been demonstrated to lie, and my first thought was that, like several of the eye-color factors of Drosophila, these two color factors were perhaps allelomorphic to each other (SHULL 1921b). This was found, however, to be a wholly erroneous guess, and another quite unexpected relationship between the two colors was discovered, for all the F_1 hybrids between these two types have had the typical yellow flowers characteristic of wild Oenothera species, generally. Crosses between homozygous *vetaurea* and homozygous *sulfurea* are given in table 6, where it is seen that sixteen matings of this type have yielded 2026 yellow-flowered offspring, and none of any other color. I believe this is the first clear case of complementary factors which has been reported in the Oenotheras.

TABLE 6

Families produced by crossing together homozygous vetaurea and homozygous sulfurea. See also table 10.

PEDIGREE NUMBERS			SEEDS SOWN	PLANTS SECURED	PERCENTAGE GERMINATION	PLANTS FLOWERED ALL YELLOW
Parents ♀	Parents ♂	Family				
2047(39)	20250(69)	21120	71	11	15.50	10
2047(39)	20241(101)	21121	300	147	49.00	141
2047(39)	20216(5)	21122	300	139	46.33	134
2047(39)	20258(75)	21124	323	127	39.32	116
20258(111)	2047(39)	21125	300	206	68.67	197
2123(8)	21254(88)	22118	300	183	61.00	91
21225(42)	21113(5)	22149	300	215	71.67	208
22167(41)	22294(49)	2399	300	34	11.33	21
22294(49)	22167(41)	23100	300	192	64.00	186
2390(5)	23141(118)	2482	300	141	47.00	137
23141(118)	2390(5)	2483	300	105	35.00	104
23141(118)	23135(5)	2487	300	148	49.33	145
23141(118)	23144(30)	2488	300	150	50.00	149
23141(118)	23147(92)	24107	300	140	46.67	140
23137(92)	23179(41)	24113	300	116	38.67	64
23141(118)	23137(80)	24123	300	216	72.00	183
Total						2026

FORMULATION OF GENETIC FACTORS FOR FLOWER COLOR

When only two flower colors, yellow and sulfur, were known in the Oenotheras, it was possible to formulate mnemonic symbols for the flower-color factors in two ways, either allowing *Y* to represent the yellow and

y the sulfur factor, or letting *S* be the yellow and *s* the sulfur factor. As the former represents the older method, it has commended itself to many geneticists for use except when special considerations render it inappropriate. With the finding of a second factor recessive to yellow, it becomes obvious that the *Vy* formulation is untenable, since *y*, which represents sulfur in one series of crosses, would represent old-gold in another series. Since there is no one factor for yellow, but two or more (possibly many) factors which, acting jointly, produce the yellow flower color, there is no reason for adopting the symbol *V* as the designation of any one factor. The only alternative, if the conspicuous advantages of mnemonic symbols are to be retained, is to choose the symbol with reference to the recessive trait, as has long been the practice of the students of Drosophila genetics, and of some others, and as has been recommended by the Committee on Genetic Form and Nomenclature, of the AMERICAN SOCIETY OF NATURALISTS (LITTLE 1921). The advantages of choosing symbols on the basis of the recessive traits, from the very start of genetical experiments with any organism, is becoming increasingly obvious, because continued work with the given organism is almost certain to show that what appeared at first to be simple characters, are in fact the resultant of the interactions of two or more genes. Symbols must then be changed to fit the new knowledge, whereas, if the recessive trait is used as the basis of the formulation, the subsequent discovery of new recessive components of a given complex are accommodated by merely adding the new symbols required, without the need of altering the symbolization applied to previously discovered elements of the complex. Adopting, then, the symbols, *s* and *S* for the *sulfurea* factor and its dominant allelomorph, the *vetaurea* factor may be represented by *v* and its dominant allelomorph by *V*. A homozygous yellow will then have the formula (with respect only to the known factors), *SSVV*; homozygous sulfur will be *ssVV*; and homozygous old-gold will be *SSvv*.

THE ORIGIN OF GOLD-CENTER, THE FOURTH FLOWER COLOR IN OENOTHERA

One of the most interesting new problems which presents itself when two factors are found to be complementary to each other, is the character to be expected in the double-recessive (in this case the *vvss*) segregate. In many cases of complementary flower colors the double recessive is phenotypically indistinguishable from one of the singly recessive traits, giving rise to the phenotypic ratio 9 : 3 : 4, and it could not be foreseen in the present case whether the double recessive would be sulfur-colored, old-gold-colored, or of an entirely new color type, and if the last should

prove true, the nature of the new color type could not be predicted. Would it be a pure white, as has often proved true in other plants, or would it have some other color?

This interesting question was not decided until late in the summer of 1923, when it was recognized that a plant in family 22197, which had been on a previous occasion recorded as *vetaurea*, was really of a new color type which has since been designated "gold-center" (seg. *aurata*). A search was at once begun for other plants of this new type, and three additional plants of *aurata* were found in family 22197, one in 22171 and two in 22199 (see table 7). Unfortunately, some of the plants in these families had already passed out of bloom, so that it was impossible to check up the classification of all those plants which had been recorded as old-gold or sulfur on previous occasions. It is possible, therefore, that several other *aurata* plants should have been added to those recorded in table 7.

The gold-center flowers have the outer portion of the petals nearly as pale as in sulfur, but of a delicate creamy-white rather than the yellowish-white of sulfurea; the center of the flower is of about the quality of the old-gold flowers (approaching Primuline Yellow) instead of the greenish-yellow center characteristic of sulfur flowers, (see plate 1).

As the flowers fade, it becomes increasingly difficult to distinguish the several color types, but there is never any difficulty in distinguishing them when the flowers are fresh, and experience has shown that fully developed buds give decisive criteria even in the absence of fresh flowers, so that classifications can now be made with full assurance that no unavoidable mistakes are made.

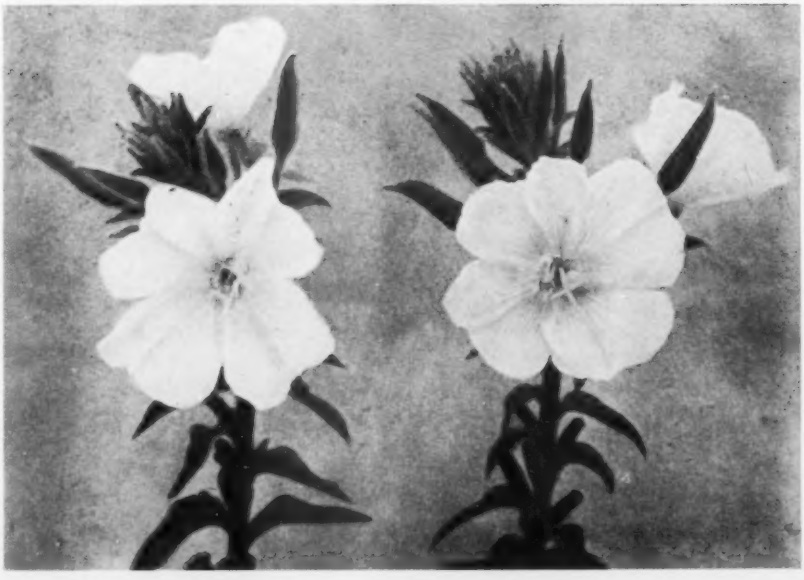
On examining the families included in table 7 it is seen that in only four of them (22171, 22197, 2396 and 2397) is there a close approximation to the typical dihybrid ratio, 9 : 3 : 3 : 1. In all the other families the *sulfurea* type is either completely absent or is present in numbers very much below what would be expected if no gamete lethals had been brought into these crosses from the sulfur-flowered grandparent. In order to properly interpret these segregations, it must be kept in mind that the lethals are linked with the *Ss* factors, which are involved in the yellow and *aurata* phenotypes as well as in *sulfurea*, so that when first-chromosome gamete lethals have accompanied the *s* factor, the ratios will be modified by the complete or partial elimination of both *sulfurea* and *aurata*, and if it has accompanied the *S* factor the percentage of yellow will be reduced.

The manner in which these "gamete lethals" produce their characteristic effects is not known, and it may, in fact, turn out that the word "lethal" is in this case a misnomer. This has been recognized from the start and

Legend for Plate 1

The four flower-color types now known in Oenothera. These are, in order, from upper left to lower right, yellow, old-gold, sulfur and gold-center. These figures have been engraved from autochrome photographs, and were all taken from segregates in family No. 24164 which consisted of 57 yellow : 53 old-gold, 48 sulfur : 55 gold-center (see table 16). The engraver has experienced considerable difficulty in reproducing these colors, and it should be noted that the color of the yellow type is distinctly more brilliant than here shown. The correct color being Ridgeway's citron yellow or nearly pale lemon yellow shading to about strontiun yellow at the center. The color of sulfur is approximately correct, being nearly napthaline yellow shaded to citron yellow or strontiun yellow at the center. In old-gold and gold-center, the yellow of the distal portion of the petals is not quite so bright yellow as here shown, being nearer Naples yellow and cream respectively; while the warmer tone toward the center of both of these latter forms approaches primuline yellow being quite noticeably less roseate than here shown. The deviations from the correct color tones are not so great, however, as to entirely destroy the usefulness of this plate.

Yellow Old-gold



Sulfur Gold-center

the word has been used because of its convenience, and always with the mental reservation that the expression is in a sense figurative, and not necessarily accurately descriptive. It has been intended only to symbolize by the expression, "gamete lethals," genetic factors which make it impossible for the egg or sperm which possesses them, to function in fertilization.

If only the yellow and *vetaurea* columns of table 7 are considered, it is seen that there are here also considerable irregularities, some of which are undoubtedly due to inadequate numbers, but for others no satisfactory explanation is yet available. Only further investigations may be competent to explain the considerable deviations from the 3 : 1 ratio in the different families. The totals of these two columns agree closely with the expected 3 : 1 ratio of yellow to *vetaurea*, the summation giving 747 yellow to 229 *vetaurea*, while the theoretical distribution would be 732 yellow to 244 *vetaurea*.

TABLE 7

Families from self-fertilized heterozygotes from crosses between vetaurea and sulfurea.

PEDIGREE NUMBERS		SEEDS SOWN	PLANTS SECURED	PERCENTAGE GERMINATION	PLANTS FLOWERED			
Parent	Family				Yellow *SV*	*Vetaurea* S_7	*Sulfurea* *sV*	*Aurata* *ss*
21120(4)	22170	300	73	24.33	21	11	2	–
21120(5)	22171	300	185	61.67	59	20	20	1
21121(24)	22176	300	76	25.33	18	1	–	–
21121(71)	22179	300	120	40.00	23	2	3	–
21122(35)	22182	300	139	46.33	38	1	–	–
21122(96)	22183	300	150	50.00	69	19	3	–
21124(29)	22191	300	106	35.33	40	20	–	–
21124(91)	22192	300	94	31.33	43	1	–	–
21125(21)	22197	300	124	41.33	40	8	5	6
21125(71)	22198	300	210	70.00	116	41	5	–
21125(170)	22199	300	253	84.33	150	60	3	2
22149(5)	2396	300	43	14.33	23	5	5	3
22149(191)	2397	300	184	61.33	80	35	20	5
23143(27)	24191	300	84	28.00	27	5	–	–

Two of the families (2396 and 2397) which gave approximately the typical dihybrid ratio, 9 : 3 : 3 : 1, are of special interest because they were derived from a cross between a strain of *Oe. franciscana sulfurea* which was thought to possess no gamete lethals and *Oe. decipiens vetaurea* which is believed to contain neither of the zygote lethals of *Oe. Lamarckiana* from which it has been indirectly derived. The near agreement of these ratios with those of typical Mendelian hybrids tends to confirm the assumptions made regarding the absence of lethals from the two P_1 plants 21225(42) and 22113(5). A more comprehensive study of this

question will be presented in a later section (page 225). Whether lethals were present in the other crosses in this table had not been predictable.

BACKCROSSES OF DIHETEROZYGOUS YELLOW, *SsVv*, TO OLD-GOLD

The discovery of the compound nature of the yellow flower color has opened up a number of new problems which will be considered in the following sections. For instance, it is important to know whether the synthesized yellow has any peculiarities of genetical behavior, as compared with the homozygous yellow of the wild type. Backcrosses to the several recessive types give the best means of testing this question. In table 8 are given the results of crossing nine diheterozygous yellow-flowered plants with a pure *Lamarckiana vetaurea* pollinator. In terms of the formulation here adopted these crosses represent the combination $SsVv \times SSvv$, and should theoretically yield 1 : 1 ratios of yellow ($SSVv + SsVv$) to *vetaurea* ($SSvv + Ssvv$). It is seen that, on the average, this expectation is closely approximated, for the totals of the nine matings consist of 337 yellow to 363 *vetaurea*, as compared with 350 of each type theoretically expected. When attention is given, however, to the individual families, it is seen at once that there are, in a number of these families, too great discrepancies between the observed and the expected ratios, to be explained by errors of random sampling. What the nature of the disturbing factors may be is not yet known.

The two *sulfurea* individuals in family 22194 may be plausibly attributed to an accident of technique which resulted in a self-fertilization of the diheterozygous mother, 21124(91).

BACKCROSSES OF DIHETEROZYGOUS YELLOW, *SsVv*, TO SULFUR

The corresponding backcrosses of the F_1 hybrids between sulfur and old-gold, to *sulfurea*, are given in table 9. These crosses may be expressed by the formula $SsVv \times ssVV$. The expectation parallels that of the preceding section, except that the 50 percent non-yellow are, in the present case, sulfur instead of old-gold. The yellow segregates should be genotypically of two sorts ($SsVV$ and $SsVv$), and the *sulfurea* segregates likewise of two genotypes ($ssVV$ and $ssVv$). If we separate out three of the eleven families in table 8 (22174, 22196 and 22202) which show rather conspicuous evidence of the effects of gamete lethals, most of the remaining families deviate but little from the expected 1 : 1 ratios, but the totals for these eight families, 496 yellow and 421 sulfur, still deviate too much from the 458.5 of each required by the 1:1 ratio.

TABLE 8

The yellow-flowered F_1 plants from crosses of vetaurea and sulfurea, backcrossed to vetaurea.

PEDIGREE NUMBERS			TYPE OF CROSS	SEEDS SOWN	PLANTS SECURED	PERCENTAGE GERMINATION	PLANTS FLOWERED		
Parents ♀	Parents ♂	Family					Yellow	*Vetaurea*	*Sulfurea*
21120(4)	21108(19)	22172	(V×S)×V	190	74	38.95	5	2	–
21120(5)	21108(19)	22173	(V×S)×V	300	99	33.00	24	13	–
21171(71)	21108(19)	22180	(V×S)×V	300	131	43.67	43	9	–
21122(96)	21108(19)	22185	(V×S)×V	300	92	30.67	30	34	–
21124(91)	21108(19)	22194	(V×S)×V	300	210	70.00	40	32	2*
21124(29)	21108(19)	22195	(V×S)×V	150	48	32.00	3	6	–
21125(21)	21108(19)	22203	(S×V)×V	300	192	64.00	48	70	–
21125(71)	21108(19)	22204	(S×V)×V	300	216	72.00	66	90	–
21125(170)	21108(19)	22206	(S×V)×V	300	241	83.33	78	107	–
Totals							337	363	2*
Expected							*350*	*350*	–

* Probably the result of an accidental self-fertilization.

TABLE 9

The yellow-flowered F_1 plants from crosses between vetaurea and sulfurea, backcrossed to sulfurea. See also table 10.

PEDIGREE NUMBERS			TYPE OF CROSS	SEEDS SOWN	PLANTS SECURED	PERCENTAGE GERMINATION	PLANTS FLOWERED	
Parents ♀	Parents ♂	Family					Yellow	*Sulfurea*
21120(4)	21225(27)	22174	, ×S)×S	300	71	23.67	29	1
21120(5)	21225(27)	22175	(V×S)×S	300	107	35.67	31	17
21121(24)	21212(39)	22177	(V×S)×S	250	84	33.60	16	29
21121(71)	21212(39)	22181	(V×S)×S	300	105	35.00	33	27
21122(35)	21195(31)	22186	(V×S)×S	300	109	36.33	29	11
21122(96)	21195(31)	22187	(V×S)×S	300	191	63.67	58	56
21124(91)	21225(27)	22193	(V×S)×S	300	163	54.33	66	50
21124(29)	21225(27)	22196	(V×S)×S	300	77	25.67	38	2
21125(21)	21225(27)	22202	(S×V)×S	300	191	63.67	9	96
21125(71)	21225(27)	22205	(S×V)×S	300	282	94.00	146	127
21125(170)	21225(27)	22207	(S×V)×S	300	243	81.00	117	104
Totals							572	520
Expected (1 : 1)							*546*	*546*

TESTS OF OLD-GOLD- AND SULFUR-FLOWERED SEGREGATES IN F_2 FAMILIES FROM CROSSES BETWEEN OLD-GOLD AND SULFUR

An interesting corollary of the Mendelian interpretation of the distribution of the flower-color factors here under consideration, is the inference that, whereas the sulfur and old-gold types were known heretofore only in the homozygous state, *ssVV* and *SSvv*, respectively, the segregates having these two phenotypic characters should be divisible in each case into homozygous and heterozygous classes with respect to their genotypic constitutions, the sulfur-flowered plants being of the two genotypes, *ssVV* and *ssVv*, and the old-gold of the two genotypes *SSvv* and *Ssvv*.

In order to discover whether these new heterozygous *sulfurea* and *vetaurea* classes were actually present among the segregates from the crosses between old-gold and sulfur, eight *vetaurea* segregates in families 22198 and 22199 were self-fertilized and also crossed with pollen from an *Oe. franciscana sulfurea* (22294(79)), and six *sulfurea* segregates in families 22194, 22198 and 22199 were self-fertilized and at the same time crossed with pollen from an *Oe. Lamarckiana vetaurea* (2220(40)). The results of these fourteen double tests are assembled in table 10, grouped according to the demonstrated genotypes. Of the 8 *vetaurea* segregates tested, 3 proved to be homozygous (*SSvv*) yielding only *vetaurea* when selfed (the unexpected presence of 13 yellows in family 23111 being certainly due to some technical error), and when crossed with homozygous sulfur produced only yellow-flowered offspring.

The remaining 5 *vetaurea* plants tested were found to be heterozygous (*Ssvv*), splitting to *vetaurea* and *aurata* when selfed and giving yellow and *sulfurea* in approximately equal numbers when crossed with homozygous sulfur. In all of the selfings of this group of plants there was a very marked deficiency in the number of *aurata* plants, the totals showing 692 *vetaurea* to 13 *aurata*. This is interpreted as meaning that the *sulfurea* factor (*s*) in all of these five plants was associated with a sperm lethal. That there was no egg lethal present is shown by the fact that the crosses to sulfur gave a total of 437 yellow to 445 sulfur, a very close fit to the expected 1 : 1 ratio.

Of the five sulfur-flowered segregates tested, two proved to be homozygous (*ssVV*) (third section of table 10), giving only sulfur when selfed and only yellow when crossed with *vetaurea*, while four (fourth section of table 10) were found to be heterozygous (*ssVv*), giving both sulfur and aurata when selfed, and both yellow and *vetaurea* when backcrossed to *vetaurea*.

The self-fertilizations totalled 422 *sulfurea* : 94 *aurata*, or 4.5 : 1, which bears the same relation to 3 : 1 that is often seen in other cases, in which an adequate explanation is found in the assumption that the recessive is slightly less effective, physiologically, than the dominant type. The backcrosses of these same plants, however, show such an excess of *vetaurea* over yellow in all families except that derived from 22194(53), that we must assume that the inadequacy of the sulfur type is a gametic rather than a somatic one, or possibly that it is both gametic and somatic.

Notwithstanding such important discrepancies as these, it is seen that this series of tests demonstrates the occurrence of heterozygous as well as homozygous *vetaurea* and *sulfurea* individuals in the progenies of the diheterozygous yellows, and the numbers of these several types,—3 *SSvv* : 5 *Ssvv*, and 2 *ssVV* : 4 *ssVv*,—are as close as possible to the 1 : 2 ratios expected.

GOLD-CENTER BREEDS TRUE

The finding of the new flower color, "gold-center," (seg. *aurata*) gave the basis of a new set of analyses of the flower color characters. These have not yet been carried out so extensively as have the breedings with old-gold, but they are, nevertheless, sufficiently consistent that there can be no doubt as to the validity of the conclusions to which they lead. It was assumed, at once, on the discovery of the gold-center plants, that they were the double-recessive segregates (*ssvv*) from the cross between sulfur and old-gold, and the correctness of this assumption has been demonstrated by the series of tests discussed in the last section and recorded in table 10. It was also recognized, however, that no other new character which originated as a definite segregate from a previous cross has been reported in the Oenotheras, and it has seemed desirable, therefore, to submit the new type to a full series of breeding tests in combination with the other color types. In table 11 it is seen that four families produced by self-fertilization of plants of the *aurata* or gold-center type yielded 489 *aurata* offspring, and none of any other color. This result agrees with what is expected of recessive types, generally, on being self-fertilized.

RECESSIVENESS OF GOLD-CENTER TO ALL OTHER COLORS

When gold-center is crossed with homozygous strains of yellow, sulfur or old-gold, it is found to be in all cases completely recessive. Table 12 gives the results of five crosses with homozygous yellow, which yielded a total of 805 yellow-flowered progeny and 1 *aurata*, the last being un-

TABLE 10

Self-fertilizations of segregates in F_2 families from crosses between velaurea and sulfurea, and backcrosses of the same plants to sulfurea and velaurea, respectively, to discover the several genotypes present. Arranged in four sections according to the demonstrated genotype.

PEDIGREE NUMBERS			TYPE OF CROSS	SEEDS SOWN	PLANTS SECURED	PERCENTAGE GERMINATION	PLANTS FLOWERED			
Parents ♀	Parents ♂	Family					Yellow	*Velaurea*	*Sulfurea*	*Aurata*
22198(15)	22198(15)	23111	V×self	300	138	46.00	13*	94	–	–
22198(15)	22294(79)	23112	V×S	300	235	78.33	205	–	–	–
22199(197)	22199(197)	23127	V×self	300	234	78.00	–	226	–	–
22199(197)	22294(79)	23128	V×S	300	157	52.33	146	–	–	–
22199(238)	22199(238)	23129	V×self	300	5	1.67	–	5	–	–
22199(238)	22294(79)	23130	V×S	300	156	52.00	150	–	–	–
Totals (3 individuals)			V×self (omitting 13 yellow in 23111)				–	325	–	–
			V×S				501	–	–	–
Demonstrated genotype, *SSvv*										
22198(38)	22198(38)	23113	V×self	300	202	67.33	–	169	–	2
22198(38)	22294(79)	24114	V×S	300	254	84.67	112	–	130	–
22198(43)	22198(43)	23115	V×self	300	75	25.00	–	62	–	–
22198(43)	22294(79)	23116	V×S	300	176	58.67	75	–	83	–
22198(55)	22198(55)	23117	V×self	300	146	48.67	–	108	–	1
22198(55)	22294(79)	23118	V×S	300	171	57.00	75	–	89	–
22199(96)	22199(96)	23123	V×self	300	244	81.33	–	186	–	5
22199(96)	22294(79)	23124	V×S	300	201	67.00	112	–	75	–
22199(162)	22199(162)	23125	V×self	300	198	66.00	–	167	–	5
22199(162)	22294(79)	23126	V×S	300	135	45.00	63	–	68	–
Totals (5 individuals)			V×self				–	692	–	13
			V×S				437	–	445	–
Demonstrated genotype, *Ssvv*										
22194(8)	22194(8)	23101	S×self	300	264	88.00	–	–	127	–
22194(8)	2220(40)	23102	S×V	300	151	50.33	110	–	–	–
22199(16)	22199(16)	23131	S×self	300	95	31.67	–	–	90	–
22199(16)	2220(40)	23132	S×V	300	167	55.67	151	–	–	–
Totals (2 individuals)			S×self				–	–	217	–
			S×V				261	–	–	–
Demonstrated genotype, *ssVV*										
22194(53)	22194(53)	23103	S×self	300	190	63.33	–	–	53	8
22194(53)	2220(40)	23104	S×V	300	92	30.67	29	14	–	–
22198(20)	22198(20)	23119	S×self	300	222	74.00	–	–	128	30
22198(20)	2220(40)	23143	S×V	300	252	84.00	89	122	–	–
22198(175)	22198(175)	23120	S×self	300	232	77.33	–	–	152	25
22198(175)	2220(40)	23121	S×V	300	230	76.67	74	104	–	–
22199(91)	22199(91)	23133	S×self	300	134	44.67	–	–	89	31
22199(91)	2220(40)	23134	S×V	300	91	30.33	31	54	–	–
Totals (4 individuals)			S×self				–	–	422	94
			S×V				223	294	–	–
Demonstrated genotype, *ssVv*										

*Undoubtedly due to some technical error.

TABLE 11

Self-fertilizations of the new flower color, gold-center (aurata).

PEDIGREE NUMBERS			SEEDS SOWN	PLANTS SECURED	PERCENTAGE GERMINATION	PLANTS FLOWERED, ALL *aurata*
Parents ♀	Parents ♂	Family				
22197(94)	22197(94)	23105	300	175	58.33	139
22199(33)	22199(33)	23122	300	115	38.33	93
2396(2)	2396(2)	24155	300	176	58.67	137
23133(59)	23133(59)	24187	300	166	55.33	120
Total						489

TABLE 12

Crosses of aurata to homozygous yellow.

PEDIGREE NUMBERS			SEEDS SOWN	PLANTS SECURED	PERCENTAGE GERMINATION	PLANTS FLOWERED	
Parents ♀	Parents ♂	Family				Yellow	*Aurata*
22197(94)	22293(76)	23107	300	65	21.67	63	–
22197(94)	2295(43)	23108	300	269	89.67	232	–
22197(94)	22166(7)	23109	300	76	25.33	70	–
22197(94)	22218(34)	23110	300	230	76.67	219	1*
22218(34)	22197(94)	23146	296	235	79.39	221	–
Total						805	1*

* Undoubtedly the result of an accidental self-pollination.

TABLE 13

Crosses of aurata to homozygous vetaurea.

PEDIGREE NUMBERS			SEEDS SOWN	PLANTS SECURED	PERCENTAGE GERMINATION	PLANTS FLOWERED ALL *vetaurea*
Parents ♀	Parents ♂	Family				
23137(92)	23133(59)	24108	300	153	51.00	86
23133(59)	23137(92)	24109	300	198	66.00	174
23133(59)	23144(30)	24142	300	205	68.33	199
23133(59)	23135(5)	24188	300	194	64.67	178
Total						637

doubtedly the result of an accidental self-fertilization of the *aurata* mother, 22197(94). Table 13 contains the data from four crosses of *aurata* with homozygous *vetaurea*, the total progeny consisting of 637 *vetaurea* and none of any other color. Table 14 gives the corresponding results of three crosses between *aurata* and homozygous *sulfurea*, the total offspring of which consisted of 620 *sulfurea*, and none of any other color.

This last case is particularly interesting from the fact that gold-center was originally recorded as an old-gold, and it is in danger of being occasionally confused with old-gold unless the classification is made from fresh flowers or from mature buds. Had old-gold instead of gold-center been used in the three crosses of table 14, the progenies would have consisted solely of yellow-flowered plants instead of sulfur-flowered, so that the cross to *sulfurea* may be used as a crucial test in any case in which doubt is entertained as to whether a given plant is old-gold or gold-center. As already stated, adequate experience in separating the color types renders the use of this crucial test unnecessary.

TABLE 14

Crosses of aurata to homozygous sulfurea.

PEDIGREE NUMBERS			SEEDS SOWN	PLANTS SECURED	PERCENTAGE GERMINATION	PLANTS FLOWERED ALL *sulfurea*
Parents ♀	Parents ♂	Family				
22197(94)	22294(49)	23106	300	249	83.00	227
23141(118)	23133(59)	2491	300	264	88.00	245
23133(59)	23179(41)	24143	300	179	59.67	148
Total						620

SEGREGATIONS OF GOLD-CENTER FROM ITS CROSSES WITH SULFUR

In table 15 is given the result of one self-fertilization, and five back-crosses of heterozygous sulfur, *ssVv*, to gold-center, *ssvv*. In only one case (family 24184) is there a close agreement with the expected ratio. In

TABLE 15

Segregation of aurata from crosses with sulfurea.

PEDIGREE NUMBERS			SEEDS SOWN	PLANTS SECURED	PERCENTAGE GERMINATION	PLANTS FLOWERED	
Parents ♀	Parents ♂	Family				*Sulfurea*	*Aurata*
23106(16)	23106(16)	24172	300	165	55.00	90	15
Expected (3:1)						*78.75*	*26.25*
23105(125)	23106(16)	24173	300	155	51.67	73	38
23106(16)	23133(59)	24174	300	130	43.33	54	32
23114(125)	23113(80)	24184	300	182	60.67	78	79
23114(125)	23113(179)	24185	300	133	44.33	62	48
23114(125)	23113(192)	24186	300	182	60.67	96	51
Total						363	248
Expected (1 : 1)						*305.5*	*305.5*

all other cases there is a deficiency of the gold-center class. The progeny from self-fertilization shows a deviation of 11.25 ± 2.99, and the totals from the backcrosses give a deviation of 57.5 ± 8.34. These deviations are probably not due to lethal factors, but perhaps to some slight inferiority of the recessive type, as is so often indicated in other organisms.

THE DOUBLE-DOMINANT SEGREGATES IN DIHYBRID F_2 FAMILIES

We have seen in table 7 that families 2396 and 2397 yielded a close approximation to the 9 : 3 : 3 : 1 ratio. These two families were especially interesting because they represented a cross between *decipiens vetaurea*, believed to be free from zygote lethals, and a strain of *franciscana sulfurea*, which was assumed to have lost the gamete lethals with which the *sulfurea* factor has been usually associated. The presentation of nearly typical dihybrid ratios in the second generation of this cross seemed to confirm the assumptions regarding the freedom of the two P_1 plants from lethals. As no one else has ever reported typical dihybrid ratios in Oenothera, it was thought desirable to determine experimentally whether the 9 : 3 : 3 : 1 ratio in these families has the significance that it has in typical Mendelian breeding. For this purpose an analysis of the genotypes represented in the double-dominant phenotypic class, yellow, was undertaken by means of backcrosses to the double-recessive type, gold-center. The nine individuals of the double-dominant class in the theoretical 9 : 3 : 3 : 1 ratio, consist of 1 pure double-dominant (*SSVV*), 2 monoheterozygotes with respect to the first factor (*SsVV*), 2 monoheterozygotes with respect to the second factor (*SSVv*), and 4 diheterozygotes (*SsVv*). Backcrosses of plants having these several genotypic constitutions, to the double recessive (*ssvv*), should give four kinds of families in the ratio 1 : 2 : 2 : 4.

Five yellow segregates in family 2396 and nine in family 2397 were used to pollinate the flowers on sixteen different branches of a single large *aurata* segregate, 2396(2), and a seventeenth branch of the same plant was self-fertilized (see table 11). The sixteen families derived from these backcrosses were grown during the past season (1925) and the results are given in table 16. The four sections of the table correspond with the four genotypes expected on a Mendelian basis, and the fit to the theoretical ratio, 1 : 2 : 2 : 4, is as close as possible,—much closer than could reasonably be expected in a test of only 16 individuals.

When consideration is given to the ratios in the individual families, however, irregularities are seen, very much too great to be attributed to errors of sampling, though the theoretical ratios are closely approached in a number of cases. Thus, all of the ratios of the third section (*SsVV*)

agree very well indeed with the expected 1 : 1 ratio, the deviation being 11.5 and the probable error, ±8.27. The last ratio of section 2 is sufficiently near to the 1 : 1 ratio, and at least one family (24164) in the last section shows a very close approach to the 1 : 1 : 1 : 1 ratio, a gametic ratio that, so far as I know, has never before been reported in the Oenotheras. The irregularities in the other families cannot at present be accounted for, and must await the light of a fuller analytical test. The most aberrant family (24162) is of special interest because of the suggested presence of a gamete lethal cutting down the *sulfurea* and *aurata* classes. Two of the families (24163 and 24168) in the second section also suggest a rather loose linkage between a gamete lethal and the old-gold factor, reducing the old-gold class to about half the expected size. An important point to be made, however, is, that if the mentioned discrepancies are in fact the result of lethal factors, there are no indications that the lethals which affect the *vetaurea* class are the same as those which modify the *sulfurea* class. All the evidence indicates that *vetaurea* and *sulfurea* belong to different linkage groups.

INDEPENDENCE OF THE OLD-GOLD FACTOR FROM OTHER FACTORS OF THE FIRST LINKAGE GROUP

While no extensive series of tests have been made to determine the linkage or independence of the old-gold factor, *v*, and its allelomorph *V*, in relation to other factors of linkage-group I, a number of the families listed in the foregoing tables have involved other first-chromosome factors besides the sulfur factor which was specifically under consideration. For the sake of simplicity no attention has been given to these other factors in the present discussion. It is sufficient for the present to simply cite an example or two to indicate the relation of *vetaurea* to other first-chromosome factors, and to summarize the experiences to date by the statement that thus far no situation has presented itself which would suggest a linkage between old-gold and any factor included in the first linkage group.

In 1921 a cross was made between one of the original *vetaurea* plants, 2047(39), which had the form of *Lamarckiana*, tall with green-hypanthium buds, and a *rubricalyx nanella*, 20202(17), dwarf, with red hypanthia. The F_1 family (21126) consisted of 20 tall yellow-flowered plants with *rubricalyx* buds (see table 2). One of the plants of this family 21126(5), self-fertilized, produced the F_2 family 22208 listed in table 3. The tall *versus* dwarf stems and red *versus* green hypanthia are closely linked

TABLE 16

Double-dominant yellow-flowered plants in (sulfurea×vetaurea)F_2, backcrossed to the double-recessive aurata.

PEDIGREE NUMBERS			SEEDS SOWN	PLANTS SECURED	PERCENTAGE GERMINATION	FLOWER COLORS OF OFFSPRING				GENOTYPE OF YELLOW-FLOWERED PARENT PLANTS
Parents ♀	Parents ♂	Family				Yellow	*Vetaurea*	*Sulfurea*	*Aurata*	
2396(2)	2396(12)	24158	300	196	65.33	159	–	–	–	*SSVV* (1 plant)
2396(2)	2397(8)	24163	300	140	46.67	75	43	–	–	
2396(2)	2397(57)	24168	300	173	57.67	90	44	–	–	*SSVv*
2396(2)	2397(62)	24171	300	219	73.00	97	83	–	–	(3 plants)
Total						262	170			
Expected (1 : 1)						*216*	*216*			
2396(2)	2396(13)	24159	300	211	70.33	86	–	90	–	
2396(2)	2396(40)	24161	300	140	46.67	34	–	44	–	*SsVV*
2396(2)	2397(50)	24165	300	249	83.00	103	–	105	–	(4 plants)
2396(2)	2397(60)	24169	300	177	59.00	66	–	73	–	
Total						289		312		
Expected (1 : 1)						*300.5*		*300.5*		
2396(2)	2396(1)	24156	300	144	48.00	22	20	23	14	
2396(2)	2396(7)	24157	300	255	85.00	46	40	54	61	
2396(2)	2396(15)	24160	300	194	64.67	39	35	49	20	
2396(2)	2396(41)	24162	300	153	51.00	70	26	4	3	*SsVv*
2396(2)	2397(48)	24164	300	228	76.00	57	53	48	55	(8 plants)
2396(2)	2397(51)	24166	300	173	57.67	46	28	30	12	
2396(2)	2397(54)	24167	300	216	72.00	43	32	54	27	
2396(2)	2397(61)	24170	291	198	68.04	38	34	54	30	
Total						361	268	316	223	
Expected (1 : 1 : 1 : 1)						*292*	*292*	*292*	*292*	
Difference						+69	−24	+24	−69	

together in the first chromosome, and as the two dominant factors, tall stems and *rubricalyx* buds, were brought into the cross from opposite sides, they segregated in the form of a typical repulsion-phase ratio, namely 1 tall with green hypanthium : 2 tall with red hypanthium : 1 dwarf with red hypanthium, the actual numbers being 14 : 39 : 13. The distribution of the flower colors was as follows:

	Tall green	*Tall red*	*Dwarf red*
Yellow	10	29	11
Old-gold	4	10	2

The close parallel in the two color classes is obvious. Putting all together in the form of a single ratio gives the numbers:

Observed	10 : 29 : 11 : 4 : 10 : 2
Expected (3 : 6 : 3 : 1 : 2 : 1)	*12 : 25 : 12 : 4 : 8 : 4*

The fit is remarkably close, in view of the rather small number of individuals available and the six-term phenotypic ratio.

The independence of old-gold from *rubricalyx* bud color was also shown by a cross of the same individual (21126(5)) whose self-fertilized progeny has just been considered, to a *Lamarckiana vetaurea*, 21108(19), this constituting a backcross of a plant heterozygous for yellow flowers and *rubricalyx* buds, to the double recessive with old-gold flowers and green hypanthia. The resulting family (22200) consisted of 15 *rubricalyx* yellow, 24 green-hypanthium yellow, 14 *rubricalyx vetaurea* and 13 green-hypanthium *vetaurea*, the 1 : 1 : 1 : 1 ratio expected on the basis of independence requiring 16.5 plants in each class. The value of χ^2 is 4.67 and P = 0.201.

THE INDEPENDENCE OF OLD-GOLD AND *brevistylis*

Having now shown that the *vetaurea* factor is independent of *sulfurea*, *nanella*, *rubricalyx* and the first-chromosome lethals, there remains the question as to its relation to the second-chromosome factor for short styles, *brevistylis*.

Self-fertilizations and backcrosses of plants in which both the *vetaurea* factor *v* and the *brevistylis* factor b_r were heterozygous have given a decisive answer to this question, as will be obvious from tables 17 and 18. The very close agreement with the 3 : 1 ratio in every color class in table 17 and with the 1 : 1 : 1 : 1 ratio in table 18, tells impressively the story of independence. The greatest deviation occurs in the *vetaurea* class in table 17 where it is 6.5 and the probable error ± 2.89. It will be noted that the backcrosses entered in table 18 involve the same three heterozygotes whose self-fertilization progenies are given in the last three lines of

table 17. The most interesting of these families is 24139, whose parent was heterozygous not only for *vetaurea* and *brevistylis* but also for *sulfurea*. Had no "pollen lethal" been associated with the sulfur factor in linkage-group I, the sulfur-flowered segregates should have been as numerous as the old-gold segregates and there should have been also a group of *aurata* offspring as well. That the parent of this family was producing *S*- and *s*-bearing eggs in equal numbers, in agreement with Mendelian theory, and that the sulfur factor is likewise independent of *brevistylis* was shown by a backcross of 23109(23) to the double recessive *sulfurea brevistylis*, 23179(41). The resulting family (24141) consisted of:

	Yellow		*Sulfur*		*Unclassified*	
	Long-styled	*Brevistylis*	Long-styled	*Brevistylis*	Long-styled	*Brevistylis*
Observed	59	61	42	38	6	5
Expected	*50*	*50*	*50*	*50*	-	-
Difference	+ 9	+11	− 8	−12	-	-

The value of χ^2 is in this case 8.2, giving P = .0427. This low value of P shows that the deviation is almost certainly in part due to other causes than the errors of random sampling, but the ratio between long-styled and *brevistylis* is almost exactly 1 : 1 in each color class, and this is the criterion of independence between the color factors and the style-length factors, in which we are here specifically interested. If the ratio 59 : 61: 42 : 38 be considered as a linkage ratio, it is seen that the crossover classes are actually slightly, though insignificantly, in excess of the non-crossover classes.

DISCUSSION

The data presented in the foregoing sections of this paper make it clear that the new flower-color factor, "old-gold," is distributed in heredity more nearly in accord with the typical Mendelian model, than any other Oenothera factor which has yet been studied, with exception of the *brevistylis* factor, which latter has always heretofore been the outstanding exception to the seeming perversities of the genetical situation in the Oenotheras.

That there are very serious deviations from the "expected" ratios in individual families must be conceded, and there is no inclination to minimize the importance of these discrepancies when I point out that the remarkable agreement with all the larger implications of the Mendelian scheme of distribution indicates that, compared with these fundamenta

TABLE 17

Segregations following self-fertilization in crosses involving vetaurea and brevistylis.

PEDIGREE NUMBERS		TYPE OF CROSS	SEEDS SOWN	PLANTS SECURED	PERCENTAGE GERMINATION	YELLOW		*Vetaurea*		*Sulfurea*		UNCLASSIFIED	
Parent	Family					Long-styled	*Brevi-stylis*	Long-styled	*Brevi-stylis*	Long-styled	*Brevi-stylis*	Long-styled	*Brevi-stylis*
21118(13)	22162	(VL×Ybr)F_1	300	40	13.33	14	2	3	2	–	–	–	–
21118(52)	22163	(VL×Ybr)F_1	300	173	57.67	66	19	12	2	–	–	2	–
22166(43)	23144	(VL×Ybr)F_2	300	160	53.33	78	22	19	5	–	–	6	2
23109(23)	24139	(AL×Ybr)F_1	300	210	70.00	85	38	32	6	9	2	3	2
23139(16)	24144	(YL×Vbr)F_1	300	96	32.00	38	11	7	3	–	–	–	–
23139(53)	24146	(YL×Vbr)F_1	300	58	19.33	21	9	7	0	–	–	–	–
Totals						302	101	80	18	9	2	11	4
Expected (3 : 1)						*302.25*	*100.75*	*73.5*	*24.5*	*8.25*	*2.75*	*11.25*	*3.75*

TABLE 18

Backcrosses of the diheterozygote to the double recessive in crosses involving vetaurea and brevistylis.

PEDIGREE NUMBERS			TYPE OF CROSS	SEEDS SOWN	PLANTS SECURED	PERCENTAGE GERMINATION	YELLOW		*Vetaurea*		UNCLASSIFIED	
Parents ♀	Parents ♂	Family					Long-styled	*Brevi-stylis*	Long-styled	*Brevi-stylis*	Long-styled	*Brevi-stylis*
23109(23)	23144(30)	24140	(AL×Ybr)F_1×Vbr	100	10	10.00	2	2	1	2	1	–
23139(16)	23144(30)	23145	(YL×Vbr)F_1×Vbr	300	69	23.00	18	11	9	13	1	1
23139(53)	23144(30)	24147	(YL×Vbr)F_1×Vbr	300	86	28.66	15	16	18	13	2	–
Total							35	29	28	28	4	1
Expected (1 : 1 : 1 : 1)							*30*	*30*	*30*	*30*	–	–

agreements, the deviations of ratios in single families are of minor significance. Further intensive and extensive analyses may be expected in time to differentiate between the genetic, physiological and environmental causes of these irregularities in the ratios.

The demonstration that the old-gold factor is independent of the sulfur factor and other factors linked with sulfur in linkage-group I, and that it is also independent of *brevistylis*, the "marker" of linkage-group II, indicates that it is the first-discovered factor of linkage-group III, for which it may well serve as the "marker." That it is proper to speak of these as *linkage groups*, and not merely as independent factors, has been shown by the discovery of a second factor closely linked with old-gold in group III (SHULL 1925), and I have evidence that there is a second factor in group II, linked with *brevistylis*.

The chief importance of these new discoveries in Oenothera, as in purely scientific genetical experiments, generally, is in the bearing they have as indicators of the nature of the mechanism which controls the distribution of the hereditary factors. It has been demonstrated by the work of many investigators, and by evidence from several different sides, that independent inheritance (Mendelian) results from the inclusion of the genes in different pairs of chromosomes. Correlatively, the work especially of MORGAN and his students and collaborators has proven that the linkage of factors in Drosophila depends upon the inclusion of the linked factors in a single chromosome pair, and this interpretation has been accepted for many other organisms in which linked genes have been studied by other investigators. I have accepted the probable correctness of this same interpretation as applied to the linkage phenomena in Oenothera.

GATES (1908) and DAVIS (1910) have found a telosynaptic arrangement of the chromosomes in Oenothera, and GATES (1923) holds that this fact is an obstacle to the occurrence of partial linkage in this genus. The genetical evidence of linkage with crossing over is unequivocal, however, and every season adds new and striking examples of it in my cultures.

CLELAND'S (1922, 1923, 1924, 1925, 1926) brilliant work on the chromosome groupings in Oenothera has brought to light a behavior among the chromosomes, which at once suggests the possibility that the linkages in Oenothera might be due to the cohesion of non-homologous chromosomes, and their separation during meiosis in such manner that the maternal members of the group are drawn to one pole and the paternal members to the other pole of the spindle. CLELAND finds a marked tendency for the

alternate members of the circles of cohering chromosomes to go to the same pole. If this behavior is of general occurrence it need only be assumed that the paternal and maternal chromosomes alternate with each other in the circles, to account for the formation of gametes predominantly of two kinds, the one possessing the entire maternal complex in so far as that complex is included in the chromosomes of the circle, and the other possessing the corresponding paternal complex. Crossing over would result, then, simply through an irregularity which would reverse the usual program with respect to one or more of the pairs of chromosomes included in the circle, so that one or more maternal chromosomes would migrate to the otherwise paternal pole and *vice versa*.

CLELAND has reported an amount of irregularity in the separation of the chromosomes that would apparently account for a very much greater degree of genetic variability than has been found by students of Oenothera genetics, and this is one of the difficulties, perhaps, in identifying chromosomal cohesion as the mechanism of linkage in this group.

There are several other difficulties which seem to me to stand in the way of accepting the hypothesis that the linkage phenomena in Oenothera result from the end-to-end cohesion of non-homologous chromosomes. One of these I have already pointed out, namely, that in the first linkage group there are now known about twice as many factors as there are haploid chromosomes in Oenothera. It might seem necessary on this account to assume the inclusion of more than one of these factors in a single chromosome, but this is not yet a *necessary* conclusion, since all of the known differential factors belonging to group I have never been included experimentally in any single zygote, (though there is no evidence at present that all can not be so included). Not until it can be shown that more linked factors can be carried by a single gamete than half the number of chromosomes involved in the circle, can it be *certain* that two or more of the linked factors *must* lie in a single chromosome pair. CLELAND suggests that a part of the linkage may be due to inclusion of genes in the same chromosome, and part to chromosomal cohesions. If this should prove true, an important question to be determined is what criteria can be used to distinguish between these two kinds of linkage.

Another difficulty for the chromosome-cohesion hypothesis is that the numerous 15-chromosome mutants from *Oenothera Lamarckiana* are characterized by marked differences in regard to nearly all features of the plant. DE VRIES and BOEDIJN (1923) have grouped these 15-chromosome forms into seven primary groups, corresponding with the seven haploid chromosomes, and have explained them on the basis of non-disjunction

of a different chromosome for each group. If this plausible hypothesis is correct, each of the haploid chromosomes produces strikingly differential effects on many features of the plant. If the production of crossovers is due to an aberrant distribution of whole chromosomes, and each chromosome produces manifold effects, it seems reasonable to suppose that the crossover individuals would be differentiated by other characteristics besides the one which is being followed in any particular breeding problem. Except in the case of the lethal factors this has been, quite generally, not the case; as a rule the crossover individuals are quite indistinguishable from the non-crossovers, except in regard to the specific characteristic which differentiated the P_1 individuals.

The old-gold mutation appeared in a strain of *Oenothera Lamarckiana* which CLELAND (1925) has studied and has found to possess 12 chromosomes united into a circle, with one pair free. As *Lamarckiana* is demonstrably carrying, all the time, in heterozygous state, several factors of linkage-group I, it might be assumed that these linked factors are distributed among the several chromosomes of the circle, and that the *vetaurea* factor lies in the independent pair. But *Lamarckiana* has always shown independence of the factor for *brevistylis*, which is found to be likewise independent of old-gold. With only *one* independent pair of chromosomes there would be no provision for *two* independent factors. There remains the logical possibility, of course, that a *Lamarckiana* which simultaneously segregates out both old-gold and *brevistylis* might be found to possess only ten chromosomes in the circle, and two independent pairs. This is a point which needs to be investigated.

The demonstration that old-gold represents not alone an independent factor, but a member of a third linkage group (SHULL 1925), and the hint that *brevistylis* is likewise not merely an independent factor but a member of the second linkage group, suggest another line of work which may be expected to give a crucial test of the chromosome-cohesion hypothesis of linkage. Thus far, CLELAND has reported only one case (*Oe. biennis*) in which the chromosomes are grouped into more than one circle,—and in this case he assumes that, although they are grouped into two circles, they are distributed as if they were included in a single circle (CLELAND 1926). The finding of as many groups of cohering chromosomes as there are linkage groups present in any given case, would strongly support the cohesion hypothesis, if it be admitted that the circles are oriented on the spindle independently of each other. It is clear that a close coöperation between geneticist and cytologist may promptly result in a decision between the chromosome-cohesion hypothesis of linkage and the hypothe-

sis that linkage results from the inclusion of factors in a single chromosome pair. If both of these cytological phenomena give rise to linkage, there may be as many linkage groups as the number of independent chromosomes plus the number of chromosome circles that are present, but no more. In other words there could be two linkage groups only in *Lamarckiana*, two in *biennis*, five in *rubrinervis*, seven in *blandina*, and so on. There is no evidence yet available that such differences exist in the number of linkage groups in the different taxonomic forms of Oenothera, but this may be due merely to the fact that we are still only at the beginning of an adequate study of linkage in this group.

SUMMARY

1. The new flower color, "old-gold" (mut. *vetaurea*) made its appearance in cultures of *Oenothera Lamarckiana* in 1921.

2. Appropriate crosses have demonstrated that it resulted from a gene mutation which probably occurred two years before the new characteristic became visible.

3. The old-gold factor, *v*, is recessive to yellow, and segregates from the yellow-flowered F_1 in a manner suggestive of a typical Mendelian recessive, but with considerable variation in the ratios.

4. The dominant yellow-flowered plants in the F_2 were found to consist of homozygotes and heterozygotes in about the typical Mendelian ratio, 1 : 2.

5. Old-gold is complementary to sulfur, the F_1 plants being always wild-type yellow.

6. In the F_2 from the cross between old-gold and sulfur, a fourth flower-color, gold-center (seg. *aurata*), appeared as the double recessive.

7. These results indicate that the four flower colors now known in Oenothera should be represented, when in the homozygous state, by the genotypic formulae: Yellow, *SSVV*; sulfur, *ssVV*; old-gold, *SSvv*; and gold-center, *ssvv*.

8. In some of the F_2 families the sulfur and gold-center classes were strikingly modified by the action of "gamete lethals," but in four families there was a close approach to the typical Mendelian dihybrid ratio, 9 yellow : 3 sulfur : 3 old-gold : 1 gold-center.

9. Tests of old-gold and sulfur segregates in F_2 families from the cross sulfur × old-gold showed that each of these types occurred in the heterozygous condition and in the homozygous condition in about the expected 2 : 1 ratio.

10. Analysis of the yellow-flowered group in an F_2 family, showed the presence of 1 *SSVV*, 3 *SSVv*, 4 *SsVV* and 8 *SsVv*, which is in essential agreement with the expected Mendelian ratio of 1 : 2 : 2 : 4.

11. The old-gold factor is independent in inheritance from the factor for sulfur and from the lethals and other factors associated with sulfur in linkage-group I.

12. It is also independent of *brevistylis* which has been used as the "marker" of linkage-group II.

13. It is, therefore, the first-discovered factor of linkage-group III, for which it may well serve as the "marker."

14. A brief critique is given of the hypothesis that linkage in the Oenotheras is based on the end-to-end fusion of non-homologous chromosomes to form circles (CLELAND).

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A METHOD FOR CALCULATING LINKAGE VALUES[1]

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INTRODUCTION

The calculation of linkage values in many plants is confined to F_2 distributions, due to difficulties in making backcrosses. EMERSON (1916), HALDANE (1919), and other authors have proposed methods by which the gametic ratio can be obtained from the observed phenotypic zygotic proportions and from this ratio the percentage of crossing over can be calculated. WOODWORTH (1916), COLLINS (1924), BRUNSON (1924) and

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others have modified or extended EMERSON'S method for use in special cases, involving duplicate, complementary or supplementary factors.

A study of the theoretical gametic ratios with their corresponding phenotypic zygotic ratios in a dihybrid F_2 distribution (table 1) has revealed some interesting relationships. Some of these relationships have already been worked out by the authors cited above, but most of those contained in this paper have not been published so far as I know. It seems desirable, therefore, to present these relationships together with additional formulae for measuring linkage intensities.

TABLE 1

Giving a number of different gametic series together with their corresponding theoretical F_2 phenotypic series.

GAMETIC SERIES				F_2 PHENOTYPIC SERIES				TOTAL (n)	CONSTANT (k)
r	s	s	r	AB	Ab	aB	ab		
10	1	1	10	342	21	21	100	484	14.611
9	1	1	9	281	19	19	81	400	14.48
8	1	1	8	226	17	17	64	324	14.320
7	1	1	7	177	15	15	49	256	14.125
6	1	1	6	134	13	13	36	196	13.877
5	1	1	5	97	11	11	25	144	13.555
4	1	1	4	66	9	9	16	100	13.12
3	1	1	3	41	7	7	9	64	12.5
2	1	1	2	22	5	5	4	36	11.555
1	1	1	1	9	3	3	1	16	10
1	2	2	1	19	8	8	1	36	8.888
1	3	3	1	33	15	15	1	64	8.5
1	4	4	1	51	24	24	1	100	8.32
1	5	5	1	73	35	35	1	144	8.222
1	6	6	1	99	48	48	1	196	8.163
1	7	7	1	129	63	63	1	256	8.125
1	8	8	1	163	80	80	1	324	8.098
1	9	9	1	201	99	99	1	400	8.08
1	10	10	1	243	120	120	1	484	8.066

SYMBOLS USED

The following symbols will be used:

r, the first and fourth member of the gametic series ($r=1$ in the repulsion phase).

s, the second and third member of the gametic series ($s=1$ in the coupling phase).

AB, Ab, aB and ab, theoretical values of the members of the F_2 phenotypic zygotic series.

AB_0, Ab_0, aB_0 and ab_0 observed values of the members of the F_2 phenotypic zygotic series.

n, the sum of AB, Ab, aB and ab.

n_0, the sum of AB_0, Ab_0, aB_0 and ab_0.

k, constant for a given series obtained by dividing the sum of AB and ab by n (or by dividing the sum of AB_0 and ab_0 by n_0) and multiplying the quotient by 16, (formulae 1 and 2).

E_0, the sum of AB_0 and ab_0.

M_0, the sum of Ab_0 and aB_0.

c, coefficient obtained by dividing n_0 by n.

THE CONSTANT (k)

As is shown in table 1, the value of the constant k is dependent on the relationship existing between the genetic factors concerned. If there is independent inheritance, k has a value of 10; if A and B are coupled, the value of k varies from 10 to 16, depending on the strength of the coupling; and if A and b are linked (repulsion phase), k varies from 10 to 8 depending on the strength of the linkage. If the linkage in the coupling phase is complete, k has a value of 16, but in case of complete linkage in the repulsion phase, k has a value of 8.

Figure 1 shows that as the value of r (coupling phase) increases, the value of the constant k also increases, approaching 16 as a limit. Figure 2 shows that as the value of s (repulsion phase) increases, the value of the constant k decreases, approaching 8 as a limit. Table 2 shows that when large values for r and s are assumed, the constant k approaches 16 in case of coupling and 8 in case of repulsion. With such high values for r and s the linkage is practically complete.

TABLE 2

Showing the value of k when the values of r (coupling) and s (repulsion) are extremely high.

GAMETIC SERIES				TOTAL (n)	CONSTANT (k)
r	s	s	r		
3199	1	1	3199	40,960,000	15.99500078125
1	3199	3199	1	40,960,000	8.00000078125

FORMULAE

The formulae given below are applicable when deviations from a dihybrid Mendelian F_2 distribution are caused by linkage. When such disturbances as differential viability of gametes or failure of many seeds of certain types to germinate are operating, the results obtained by the application of the formulae to observed values will be misleading. The

following formula shows the method of obtaining the value of k from the theoretical F_2 phenotypic series:

$$k=\frac{16(AB+ab)}{n} \tag{1}$$

The general formula also holds for obtaining the value of k from observed F_2 phenotypic ratios:

$$k=\frac{16(AB_0+ab_0)}{n_0} \tag{2}$$

Most of the formulae that follow indicate the relationship of the members of *theoretical* series. Several formulae are given to show the relationship when *observed* values are used. All formulae which are applicable to observed values are applicable also to theoretical values, but formulae which are applicable to theoretical values are applicable to observed values only when the value of c is unity. The theoretical values can be calculated from the observed values by means of the constant k.

Formulae for finding the value of n when the value of r, s, AB, Ab, aB, ab, or k is known

Coupling phase

$$n=4(r+1)^2 \tag{3}$$

From formulae (15) and (17) the following value for AB is derived,

$$AB=\frac{n}{2}+\left(\frac{\sqrt{n}}{2}-1\right)^2 \tag{4}$$

Hence,

$$n=\left[\frac{2\sqrt{3AB-2}+2}{3}\right]^2 \tag{5}$$

$$n=(Ab+1)^2 \text{ or } (aB+1)^2 \tag{6}$$

$$n=\left[2(\sqrt{ab}+1)\right]^2 \tag{7}$$

$$n=\left[\frac{4(\sqrt{2k-16}+4)}{16-k}\right]^2 \tag{8}$$

Repulsion phase

$$n=4(s+1)^2 \tag{9}$$

$$n=2(AB-1) \tag{10}$$

$$n=4(Ab+1) \tag{11}$$

$$n=\frac{32}{k-8} \tag{12}$$

Formulae for finding the value of any member of either gametic or F_2 phenotypic series when the value of n is known

Coupling phase

$$r=\frac{\sqrt{n}}{2}-1 \tag{13}$$

$$AB=\frac{3n-4(\sqrt{n}-1)}{4} \text{ (derived from formula 5)} \tag{14}$$

$$AB=\frac{n}{2}+ab \tag{15}$$

$$Ab \text{ or } aB=\sqrt{n}-1 \tag{16}$$

$$ab=\left[\frac{\sqrt{n}}{2}-1\right]^2 \tag{17}$$

$$k=\frac{16\left[(\sqrt{n}-1)^2+1\right]}{n} \tag{18}$$

Repulsion phase

$$s=\frac{\sqrt{n}}{2}-1 \tag{19}$$

$$AB=\frac{n}{2}+1 \tag{20}$$

$$Ab \text{ or } aB=\frac{n}{4}-1 \tag{21}$$

$$k=\frac{8(n+4)}{n} \tag{22}$$

Formulae showing the relation of the different members of the phenotypic series to one another

Coupling phase

Substituting in formula 4, the value of n obtained in formula 6, we have

$$AB=\frac{(Ab+1)^2}{2}+\left[\frac{Ab+1}{2}-1\right]^2$$

which reduces to

$$AB=\frac{3\,(Ab)^2+2Ab+3}{4} \tag{23}$$

Substituting in formula 4, the value of n obtained in formula 7, we have

$$AB=\frac{[2(\sqrt{ab}+1)]^2}{2}+\left[\frac{2\sqrt{ab}+2}{2}-1\right]^2$$

which reduces to

$$AB=3ab+4\sqrt{ab}+2 \tag{24}$$

$$Ab=\frac{2\sqrt{3AB-2}-1}{3}\ \text{(derived from formula 23)} \tag{25}$$

Substituting in formula 16, the value of n obtained in formula 7, we have

$$Ab=2\sqrt{ab}+1 \tag{26}$$

Substituting in formula 17, the value of n obtained in formula 5, we have

$$ab=\left[\frac{\sqrt{3AB-2}+1}{3}-1\right]^2$$

which reduces to

$$ab=\frac{3AB-4\sqrt{3AB-2}+2}{9} \tag{27}$$

Substituting in formula 17, the value of n obtained in formula 6, we have

$$ab=\left[\frac{Ab+1}{2}-1\right]^2$$

which reduces to

$$ab=\left[\frac{Ab-1}{2}\right]^2 \tag{28}$$

Repulsion phase

$$AB=2Ab+3 \tag{29}$$

Formulae for finding the percentage of crossing over when the value of r, s, AB, Ab, aB, ab, n or k is known

Coupling phase

$$\text{Percentage of crossing over}=100\left[\frac{1}{r+1}\right] \tag{30}$$

From formulae 3 and 4 we derive

$$AB=\frac{4(r+1)^2}{2}+\left[\frac{2(r+1)}{2}-1\right]^2$$

From this equation we obtain

$$r=\frac{\sqrt{3AB-2}-2}{3} \tag{31}$$

Substituting in formula 30, the value of r obtained in formula 31, we have

$$\text{Percentage of crossing over} = 100\left[\frac{3}{\sqrt{3AB-2}+1}\right] \quad (32)$$

$$r = \frac{Ab-1}{2} \quad \text{(derived from formulae 3 and 6)} \quad (33)$$

From formulae 30 and 33 we derive

$$\text{Percentage of crossing over} = 100\left[\frac{2}{Ab+1}\right] \quad (34)$$

$$r = \sqrt{ab} \quad \text{(derived from formulae 3 and 7)} \quad (35)$$

From formulae 30 and 35 we derive

$$\text{Percentage of crossing over} = 100\left[\frac{1}{\sqrt{ab}+1}\right] \quad (36)$$

$$\text{Percentage of crossing over} = 100\left[\frac{2}{\sqrt{n}}\right] \quad \text{(derived from formulae 3 and 30)} \quad (37)$$

From formula 8 we derive

$$r+1 = \frac{2(\sqrt{2(k-8)}+4)}{16-k} \quad (38)$$

Hence, $$\text{Percentage of crossing over} = 100\left[\frac{16-k}{2\sqrt{2(k-8)}+8}\right] \quad (39)$$

Repulsion phase

$$\text{Percentage crossing over} = 100\left[\frac{2}{\sqrt{2(AB-1)}}\right] \quad (40)$$

$$\text{Percentage crossing over} = 100\left[\frac{1}{\sqrt{Ab+1}}\right] \quad (41)$$

$$\text{Percentage crossing over} = 100\left[\frac{2}{\sqrt{n}}\right] \quad (42)$$

$$\text{Percentage crossing over} = 100\left[\frac{k-8}{2\sqrt{2(k-8)}}\right] \quad (43)$$

Formulae for calculating the percentage of crossing over from observed values

Coupling phase

Substituting in formula 39, the value of k obtained in formula 2, we have

$$\text{Percentage crossing over} = 100\left[\frac{\left(16-\frac{16(AB_0+ab_0)}{n_0}\right)}{2\sqrt{2\left(\frac{16(AB_0+ab_0)}{n_0}\right)-16+8}}\right]$$

which is equivalent to

$$\text{Percentage crossing over} = 100\left[1-\sqrt{\frac{AB_0-Ab_0-aB_0+ab_0}{AB_0+Ab_0+aB_0+ab_0}}\right]$$

Substituting E_0 for the sum of AB_0 and ab_0, M_0 for the sum of Ab_0 and aB_0, and n_0 for the sum of AB_0, Ab_0, aB_0 and ab_0, we have,

$$\text{Percentage of crossing over} = 100\left[1-\sqrt{\frac{E_0-M_0}{n_0}}\right] \tag{44}$$

Repulsion phase

Substituting in formula 43, the value of k obtained in formula 2, we have

$$\text{Percentage crossing over} = 110\left[\frac{\frac{16(AB_0+ab_0)}{n_0}-8}{2\sqrt{2\left(\frac{16(AB_0+ab_0)}{n_0}-8\right)}}\right]$$

which is equivalent to

$$\text{Percentage crossing over} = 100\sqrt{\frac{AB_0-Ab_0-aB_0+ab_0}{AB_0+Ab_0+aB_0+ab_0}}$$

Substituting E_0 for the sum of AB_0 and ab_0, M_0 for the sum of Ab_0 and aB_0, and n_0 for the sum of AB_0, Ab_0, aB_0 and ab_0, we have

$$\text{Percentage crossing over} = 100\sqrt{\frac{E_0-M_0}{n_0}} \tag{45}$$

The percentage of crossing over may also be obtained by dividing E_0 by M_0 and referring to table 3. Fractional percentages can be obtained by interpolation. As an example, the percentage of crossing over may be calculated as follows from the first distribution in table 4:

$$E_0 = 631; \qquad M_0 = 50$$

$$\frac{E_0}{M_0} = 12.6$$

By referring to table 3 we find that when the value of $E_0 \div M_0 = 13.8038$ the percentage of crossing over is 7 and when the value of $E_0 \div M_0 =$ 12.0208 the percentage of crossing over is 8. The difference = 1.7830

$$13.8038 - 12.6 = 1.2038$$

$$\frac{1.2038}{1.7830} \text{ of 1 per cent} = 0.67 \text{ per cent}$$

$$7.0 \text{ per cent} + 0.67 \text{ per cent} = 7.67 \text{ per cent}$$

TABLE 3

Crossing-over percentages from 1 to 50 and the corresponding ratios of E_0 *to* M_0.

COUPLING PHASE				REPULSION PHASE			
Percent crossing over	$\frac{E_0}{M_0}$	Percent crossing over	$\frac{E_0}{M_0}$	Percent crossing over	$\frac{E_0}{M_0}$	Percent crossing over	$\frac{E_0}{M_0}$
1	99.5025	26	3.4209	1	1.0002	26	1.1450
2	49.5051	27	3.2817	2	1.0008	27	1.1573
3	32.8409	28	3.1528	3	1.0018	28	1.1701
4	24.5102	29	3.0331	4	1.0032	29	1.1836
5	19.5128	30	2.9215	5	1.0050	30	1.1978
6	16.1822	31	2.8175	6	1.0072	31	1.2126
7	13.8038	32	2.7202	7	1.0098	32	1.2282
8	12.0208	33	2.6291	8	1.0129	33	1.2444
9	10.6346	34	2.5436	9	1.0163	34	1.2614
10	9.5263	35	2.4631	10	1.0202	35	1.2792
11	8.6200	36	2.3875	11	1.0245	36	1.2978
12	7.8652	37	2.3162	12	1.0292	37	1.3172
13	7.2271	38	2.2488	13	1.0344	38	1.3375
14	6.6805	39	2.1852	14	1.0400	39	1.3588
15	6.2072	40	2.1250	15	1.0460	r0	1.3810
16	5.7935	41	2.0679	16	1.0525	41	1.4041
17	5.4288	42	2.0139	17	1.0595	42	1.4283
18	5.1050	43	1.9625	18	1.0670	43	1.4537
19	4.8157	44	1.9137	19	1.0749	44	1.4802
20	4.5556	45	1.8674	20	1.0833	45	1.5078
21	4.3206	46	1.8232	21	1.0923	46	1.5368
22	4.1073	47	1.7813	22	1.1017	47	1.5670
23	3.9128	48	1.7412	23	1.1117	48	1.5988
24	3.7349	49	1.7031	24	1.1222	49	1.6319
25	3.5714	50	1.6667	25	1.1333	50	1.6667

DISCUSSION

In order to illustrate the method of application of some of the formulae, table 4 was compiled. The data in this table are the same as those used by EMERSON (1916). Formula 2 was first used to obtain the value of k. From the value of k, the value of n was calculated by using formula 8 for

the first two distributions and formula 12 for the third distribution. Finding the value of n in the coupling phase involves several computations because formula 8 is quite complex, but after n has once been obtained the calculation from n, of the other members of the zygotic series, is relatively simple. The value of k in the fourth distribution is 7.94. Since this value is less than 8, the repulsion formulae are not applicable. EMERSON (1916) found that the value of r for this distribution could not be determined by the formula which he proposed because $(1335+2)-(643+714)$ is a negative quantity (-20) and has no real root.

TABLE 4

Theoretical values of various terms obtained from observed values.

OBSERVED					THEORETICAL						
AB	Ab_0	aB_0	ab_0	n_0	k	$r:s$	AB	Ab	aB	ab	n
493	25	25	138	681	14.825	12.1 : 1	488.8	25.2	25.2	146.1	685.3
165	58	58	78	359	10.830	1.46 : 1	14.30	3.93	3.93	2.13	24.34
336	150	143	11	640	8.675	1:2.44	24.7	10.85	10.85	1	47.4
1335	643	714	2	2694	7.94	.....	.....	.....	.....	.....	.....

The formulae reported in this paper are applicable only when the value of k is between 10 and 8 (repulsion phase) or 10 and 16 (coupling phase). With a reduction of the same number of AB_0 or ab_0 types for a given value of r or s the limits for the application of the formulae are reached sooner in the repulsion phase than in the coupling phase as is indicated by the nature of the curves in figures 1 and 2. In the coupling phase the value of the points on the curve approaches 16 *gradually*, while in the repulsion phase it approaches 8 *rapidly* at first, then more slowly.

The relationship of E_0 and M_0 to r and s is shown in the following formulae:

$$\sqrt{\frac{E_0-M_0}{E_0+M_0}}=\frac{r}{r+1} \qquad \text{(coupling phase)} \tag{46}$$

and

$$\sqrt{\frac{E_0-M_0}{E_0+M_0}}=\frac{1}{s+1} \qquad \text{(repulsion phase)} \tag{47}$$

The relationship between E_0 and M_0 may also be expressed as follows:

$$E_0=M_0+4cr^2 \qquad \text{(coupling phase)} \tag{48}$$

and

$$E_0=M_0+4c \qquad \text{(repulsion phase)} \tag{49}$$

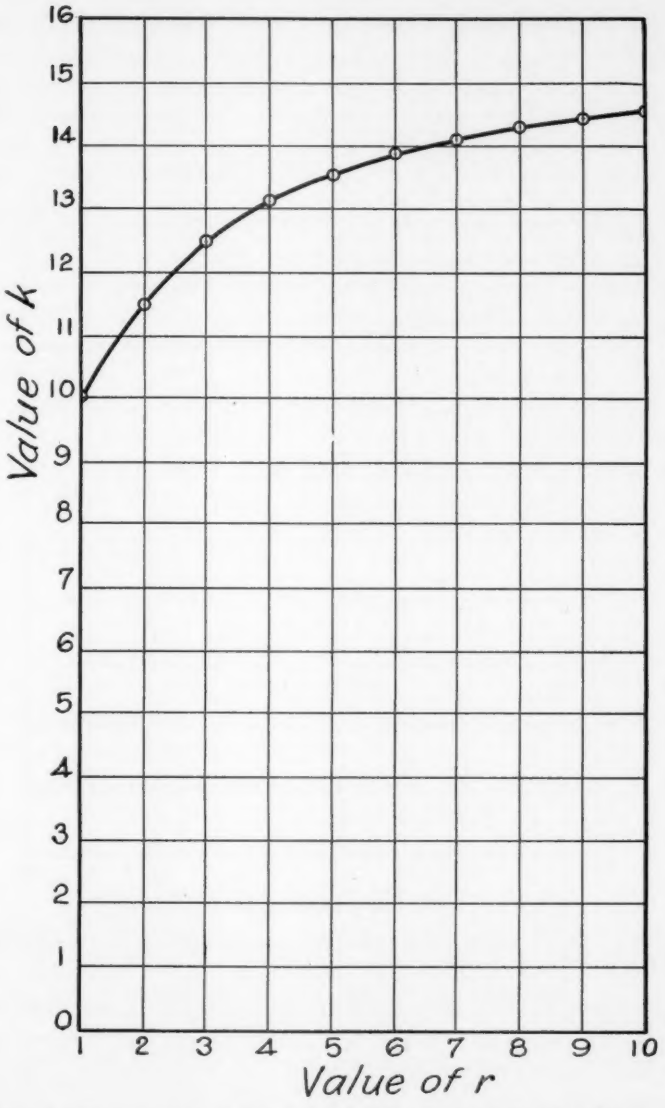

FIGURE 1.—The curve shows that when the value of r is increased, the value of k approaches 16 gradually.

A further relationship between E_0 and M_0 is indicated by formulae 50 and 51:

$$\frac{E_0}{M_0} = r + \frac{1}{2} + \frac{c}{M_0} \qquad \text{(coupling phase)} \tag{50}$$

and

$$\frac{E_0}{M_0} = 1 + \frac{4c}{M_0} \qquad \text{(repulsion phase)} \tag{51}$$

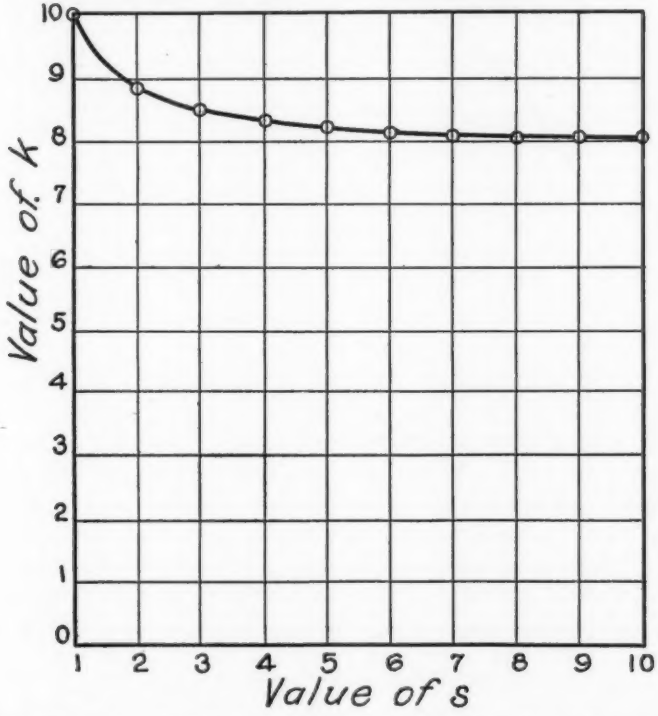

FIGURE 2.—The curve shows that when the value of s is increased, the value of k approaches 8, rapidly at first, then more slowly.

The value of E_0 divided by M_0 in the coupling phase is approximately equal to $r+\frac{1}{2}$ and the greater the magnitude of r the less is the magnitude of $\frac{c}{M_0}$ which approaches zero as the value of r approaches infinity. On the other hand, the value of E_0 divided by M_0 in the repulsion phase is approximately equal to 1, and the magnitude of $\frac{4c}{M_0}$ decreases as the

value of s increases. The value of $\frac{4c}{M_0}$ approaches zero as the value of s approaches infinity. In other words, the ratio of E_0 to M_0 is approximately equal to the magnitude of $r+\frac{1}{2}$ in the coupling phase while in the repulsion phase E_0 is approximately equal to M_0. With this relationship in mind it is evident that a considerable reduction of AB_0 and ab_0 types, caused perhaps by differential viability or selective fertilization, is permissible in the coupling phase to meet the conditions necessary for the application of the formulae. The effect of such reductions, however, is not corrected by the formulae and thus an erroneous value for r is obtained. On the other hand, owing to the approximate equality of E_0 and M_0 in the repulsion phase, a slight reduction of the AB_0 and ab_0 types may cause the value of E_0 to be less than M_0. If the reduction of these types is greater than $4c$, then E_0 minus M_0 will have a negative value.

In order that the formulae may be usable, the observed values should deviate as little as possible from the result obtained by multiplying the theoretical distribution by c. If the observed distribution corresponds to this distribution, the quotient obtained by dividing the sum of the types AB_0 and Ab_0 by the types aB_0 and ab_0 is a constant, being equal to 3 in both coupling and repulsion phases.

$$\frac{AB_0+Ab_0}{aB_0+ab_0}=3 \qquad (52)$$

SUMMARY

1. A constant k which shows the relation of observed values in linkage studies to independent inheritance is obtained.

2. The value of k indicates the following:
   (a) When $k=10$, independent inheritance.
   (b) When $k=$ more than 10 but less than 16, coupling.
   (c) When $k=$ less than 10 but more than 8, repulsion.
   (d) When $k=$ less than 8, some factor other than chance resulting in marked deviations from theoretical ratios.

3. Formulae are given showing the relation of the members of the theoretical gametic and zygotic series to one another.

4. The relationship between the extreme terms and the middle terms of a distribution is shown.

5. Formulae and tables for determining the percentage of crossing over are presented.

ACKNOWLEDGMENT

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THE LINKAGE RELATIONS OF A BENIGN TUMOR IN DROSOPHILA

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INTRODUCTION

It has already been reported (STARK 1919) that the "benign" tumor which appeared as a mutation in the lethal-tumor strain of flies (lethal 7) is due primarily to a recessive gene located in the third chromosome close to dichaete. This conclusion was based on the results of a backcross between the tumor and dichaete and hairless (P_1, *D H* ♀ × t_u ♂; F_1, *D H* ♀ × t_u ♂) which gave: *D H* = 348, wild-type = 276, *D* = 108, *H* = 78, t_u = 30, t_u *H* = 10. Instead of 50 percent, only 5 percent of the flies in the backcross showed the tumor character. This low value indicated that more than one recessive gene was concerned in the production of the tumor. In order to locate more exactly the principal gene in the third chromosome and also to locate the other genes that appeared to be involved, the same methods were employed that had been used in analyzing the complex inheritance of vortex (BRIDGES and MOHR 1919) and of ski (CLAUSEN and COLLINS 1922).

STAR DICHAETE × TUMOR, MALE BACKCROSS

From the pure-breeding stock of tumor flies two males were mated, each to a star dichaete female. Star is a second-chromosome dominant

located at the extreme left end of the second-chromosome map (0.0). Dichaete is a third-chromosome dominant located somewhat to the left of the middle of the third chromosome (40.4). The F_1 star dichaete males were then mated separately, each to a virgin female from the tumor stock. (In table 1, Nos. 11 to 18 are from one P_1 mating, and Nos. 31 to 36 are from the other.) The flies of the "backcross" generation were classified with respect to star, dichaete and tumor.

When the results for the different backcross cultures were examined, it was seen that there was a very striking diversity, not only in the percentages of tumor flies (in the last column), but also in the frequency with which star and dichaete flies bore tumors. This signified that the tumor stock or the star dichaete stock or both had not been homozygous with respect to the genes affecting tumor development. If the stocks had been uniform, all of the backcross cultures should have been of a single type, though differences due to errors of random sampling might be present. A serious source of difference in the use of certain characters arises from their extreme sensitivity to slight and uncontrolled environmental differences, such as quality and condition of the food materials. Thus, a high proportion of the Drosophila mutant characters show a progressive "changing over" toward the wild type as the flies emerge later and later in the course of the culture. This can be largely corrected by the occasional addition of fresh food early in the course of the culture, but an extended examination of the cultures producing tumors suggests that the differences in the degree of development of the character (the percentage of individuals that show the character) are in the main dependent on genetic causes rather than environmental. In the experiment whose results are given in table 1 the cultures fell into four types, each sharply different from the others, and the constituent members of a given group resembled each other closely.

The most frequent type of culture that occurred in the star dichaete ×tumor male backcross was characterized by complete or nearly complete absence of tumors in flies that were dichaete or dichaete with star (table 1, upper division). This showed that the genes (or gene) having the greatest effectiveness in the production of the tumors were recessives in the third chromosome. That is, a fly in those cultures could not develop tumors unless both third chromosomes were derived from the tumor stock, one directly from the tumor-bearing mother and the other through the F_1 star dichaete male. The third chromosome from the tumor stock that was present in the F_1 *S D* ♂ was kept intact because there is no cross-

ing over in a male, and was transmitted to all of the offspring that did not show the dominant character dichaete.

But the fact that a fly was homozygous for the entire third chromosome from the tumor stock did not insure that tumors develop in it. Thus, of the 631 flies, (of the upper division of table 1) that were not-dichaete, and that therefore had both their third chromosomes from the tumor stock, 343 or 54 percent had tumors, but the remainder, or 46 percent, did not have tumors. These 46 percent of flies were non-tumor-bearing because some other gene or genes besides the third-chromosome recessives were necessary to insure the development of the tumor character. That these other genes were predominantly in the second chromosome was shown by the fact that among the not-dichaete flies that were star, some (91) were tumored but many more (230) were non-tumored.

TABLE 1

P_1, star dichaete ♀ × tumor ♂; backcross l_u ♀ × F_1SD ♂.

GROUP	CULTURE NUMBER	+	l_u	S	S l_u	D	D l_u	S D	S D l_u	PERCENT l_u
I	11	3	37	32	13	42	..	50	..	29
	15	3	38	21	7	34	..	34	..	33
	31	..	9	10	4	11	..	20	..	24
	32	25	87	78	39	84	..	103	1	30
	35	15	54	47	14	71	..	70	2	25
	36	12	27	42	14	42	..	65	..	30
Totals		58	252	230	91	284	..	342	3	27
II	13	4	22	2	12	18	2	24	..	43
	14	1	12	3	9	13	..	13	..	41
	17	6	31	15	21	37	..	31	..	37
	33	6	17	9	12	19	..	17	..	36
Totals		17	82	29	54	87	2	85	..	40
III	12	5	13	23	2	21	6	19	1	25
	16	5	20	29	5	12	12	30	..	33
Totals		10	33	52	7	33	18	49	1	29
IV	18	2	19	6	11	5	12	10	5	66

A closer analysis of the effectiveness of the mutant genes involved could be made by studying the percentage of occurrence of tumors in the different classes with respect to star and dichaete. Thus, the class *S D*

differed from wild flies by being heterozygous for any tumor genes carried in chromosomes II and III of the tumor stock. Of the *S D* flies in group I only 3 bore tumors to 342 that did not. That is, the dominant effect of the combined second and third chromosome genes upon production of tumors was negligible. The flies that were dichaete but not star differed from the *S D* flies in that they were homozygous for the second-chromosome genes of the tumor stock. Of the *D* flies none bore tumors, while 284 were without tumors. As pointed out before, this meant that homozygosis for second-chromosome genes was ineffective if the fly were only heterozygous for the main tumor genes, which were recessives in the third chromosome. The *S* not-*D* flies differed from the *S D* flies in that they were homozygous for third-chromosome genes. Of the *S* flies 91 were tumor-bearing to 230 that were free from tumors. That is, homozygosis for the third-chromosome genes produced tumors in about a quarter of the flies that were only heterozygous for the second-chromosome genes. Conversely, the second-chromosome genes were not essential in a homozygous form, for 91 flies merely heterozygous for the second-chromosome genes were made to produce tumors by the action of the third-chromosome gene. This agreed with the previous comparison (of frequency of tumors in all *D* as compared with all not-*D* flies) in showing that the third-chromosome genes were responsible for a greater share of tumor development than were the second-chromosome genes. A comparison of the not-*S* not-*D* class with the *S* not-*D* class showed that in the presence of homozygous III tumor genes, homozygous II tumor genes raised the percentage of tumors from 28 to 81. This showed a considerable effectiveness of recessive second-chromosome genes, accounting for nearly all of the effectiveness not already accounted for by third-chromosome recessives. Finally, in flies that were homozygous for chromosome II and at the same time for chromosome III (that is, that were neither star nor dichaete) 352 were tumored and 58, or 19 percent, were non-tumored. Since all of the flies of the parental tumor stock were tumor-bearing, it follows that flies that were homozygous for both the second and third chromosomes and yet failed to develop tumors (19 percent) lacked certain tumor genes that were present in the parental stock. The locus of these other genes, of relatively slight aggregate effect, must be sought in the other chromosomes, —the X and chromosome IV.

The second group of backcross cultures (table 1, second division) agreed with the first in that practically none of the flies that were dichaete was at the same time tumor-bearing, but differed in that the star (not-dichaete) flies with tumors (54) outnumbered the star (not-dichaete) flies

without tumors (29). In the first group the incidence of tumors in the star not-dichaete flies was 28 percent, while it was 65 percent in the second group. Moreover, the percentage frequency for each culture was higher than in the first group, giving an average of 40 percent instead of 27 percent. The increase from 28 to 65 percent of tumors in the star class, and the increased frequency of tumors from 27 to 40 percent per culture, could be accounted for either by assuming that additional third-chromosome recessive genes were present in group II cultures, or that there were additional second-chromosome genes that were dominant or semi-dominant.

The third group of cultures comprised two only, which agreed with the first group with respect to the percentage frequency of tumors in the star (not-dichaete) class, but differed in that a considerable percentage of the (not-star) dichaete flies were tumor-bearing (18÷51 or 35 percent, as compared with 0÷284 in group I). This difference could be attributed either to additional second-chromosome recessives, or, more probably, to additional third-chromosome dominants.

One culture was present (group IV) that apparently combined the additional modifiers present in group II with the additional modifiers present in group III. Thus, in the star class 65 percent of the star flies were tumor-bearing, which agreed with the 65 percent of group II. In the *S D* class 35 percent of the flies were tumor-bearing, showing conclusively the presence of dominant modifiers, and agreeing with the hypothesis advanced for group III. Two additional sets of modifiers also resulted in an increase in the total tumor frequency from the 27 percent of group I to 66 in group IV.

In all of the four groups the percentage of non-tumored flies in the not-star not-dichaete class (columns 1 and 2) was approximately constant, with an average of 18 percent (I=19, II=17, III=23, IV=10). That is, the tumor stock used, that gave 100 percent of tumor-bearing flies, differed from the non-tumored star dichaete parent stock in genes outside of the controlled chromosomes II and III to the extent of 18 percent of the total tumor production. Apparently the stocks were uniform with respect to these other genes, located in the X and IV chromosomes. The stocks were also uniform with respect to the second- and third-chromosome genes responsible for the results of group I, and present in the other groups. The stocks contained, but were not uniform for, additional second- and third-chromosome modifiers responsible for group II, and for group III, and, acting together, for group IV.

STAR DICHAETE × TUMOR, FEMALE BACKCROSS

From the crosses of star dichaete × tumor that gave the F_1 males tested in table 1, star dichaete F_1 females were mated each to a male from the tumor stock. The cultures (table 2) were not markedly different from one another, ranging in percentage of tumors from 10 to 21. Three subtotals have been calculated: for the lowest three cultures; for the group having 16 to 17 percent of tumors; and for the group having 20 or 21

TABLE 2.

P_1, star dichaete ♀ × tumor ♂; backcross, F_1 S D ♀ × l_u ♂.

CULTURE NUMBER	+	l_u	S	S l_u	D	D l_u	S D	S D l_u	PERCENT l_u
4	70	28	81	12	127	5	117	1	10
3	137	58	159	21	151	5	174	3	12
1	101	40	108	28	138	7	130	1	14
Totals	308	126	348	61	416	17	421	5	12
2	123	52	121	48	195	16	146	3	16
5	85	51	123	28	148	5	138	8	16
6	104	38	118	36	128	7	100	4	16
7	56	30	60	17	75	6	86	..	16
9	68	45	68	21	123	3	87	..	16
30	46	26	55	14	76	5	66	1	16
29	43	27	52	13	44	..	61	1	17
Totals	525	269	597	177	789	42	684	17	16
8	33	32	47	13	53	5	65	..	20
26	77	52	93	26	104	11	94	4	20
27	56	37	81	30	86	4	78	..	20
28	41	32	39	13	55	2	54	4	21
25	42	40	51	22	64	1	83	2	21
Totals	249	193	311	104	362	23	374	10	20
Grand Totals	1082	588	1256	342	1567	82	1479	32	16

percent of tumors. The grand total gives 16.3 percent of tumors, which was lower than the percentage (27) given in group I of table 1. Most of the decrease occurred in the not-star not-dichaete class, in which there were 588 tumor-bearing to 1479 normal flies. The percentage of tumored flies in this class was 35 as compared with 81 percent in the same class in the reciprocal backcross.

Such a difference was to be expected if there were in the second chromosome more than one gene favoring tumor development, and likewise in the third. In the crosses of table 1 the several tumor genes in a given chromosome were held together by the lack of crossing over in the male, and consequently, could give a maximum number of favorable combinations. In the female, crossing over occurs and allows the tumor genes in a given chromosome to be separated, so that the number of favorable combinations is decreased, and is the smaller the higher the crossing over, that is, the farther apart within the chromosome the various genes lie. The observed decrease was taken to demonstrate that the effect due to the second or the third chromosome or to both was an aggregate one and not due to a single gene in each.

While crossing over tended to decrease the total production of tumors, it tended to increase the proportion of tumors that occurred with star and with dichaete. Thus, of the 1044 flies with tumors, 374 or 36 percent were with star, as compared with 27 percent with star in group I of the male backcross. Likewise 11 percent tumors were with dichaete as contrasted with 1 percent in the reciprocal backcross. The results with dichaete suggest that for the third chromosome most of the effect is due to a main gene located roughly 10 units from dichaete. For the second chromosome the effect is more widely distributed, but little can be said as to the location of the particular genes.

From table 2, a few females of the star dichaete class were mated singly to males of the tumor class (table 3). Three of the cultures gave percentages of tumors like those of table 2, but the other three gave markedly higher tumor percentages. In these last-mentioned cultures the mothers had become homozygous for certain tumor genes, either those outside chromosomes II and III, which involved simply assortment, or

TABLE 3

S D ♀ (*from table 2*)×l_u ♂ (*from table 2*).

CULTURE NUMBER	+	l_u	*S*	*S* l_u	*D*	*D* l_u	*S D*	*S D* l_u	PERCENT l_u
20	61	37	80	11	86	8	99	3	15
22	73	31	65	12	53	7	58	2	17
21	34	19	33	12	34	3	35	5	22
19	79	31	85	55	103	8	79	37	27
23	29	36	36	38	54	17	64	10	36
24	22	37	26	24	32	10	42	9	40
Totals	298	191	325	152	362	53	377	66	25

TABLE 4

P_1, tumor ♂×dichaete hairless ♂; backcross, F_1 D H ♀×t_u♂.

GENERATION	CULTURE NUMBER	*D H*	+	*D*	*H*	t_u	t_u *D H*	t_u *H*	t_u *D*	PERCENT t_u
BC	37	181	229	59	48	39	12	3	..	9
	38	155	235	68	52	27	2	4	.	6
	39	69	53	28	18	16	3	5	1	12
	40	81	109	31	25	13	1	5		7
	41	77	86	17	31	7	5	..	..	5
	Totals	563	712	203	174	102	23	17	1	8
BC_2	42	27	43	13	12	10	3	3	1	15
	43	102	142	50	38	4	..	3	2	2
	44	67	62	16	9	16	2	2	5	14
	45	54	93	26	5	25	6	2	..	15
	46	43	53	21	8	29	28	8	11	37
	47	76	63	29	12	54	17	7	10	33
	48	119	126	57	37	33	24	9	6	17
	Totals	488	582	212	121	171	80	34	35	19
BC_3	49	44	59	26	13	30	8	6	2	20
	50	30	14	15	4	21	16	10	6	46
	51	52	37	29	22	30	7	13	1	26
	52	34	51	29	13	56	33	25	10	50
	53	48	65	23	18	15	6	5	..	15
	Totals	208	226	122	70	152	70	59	19	32
BC_4	54	34	70	20	21	12	6	9	4	17
	55	33	33	30	11	17	14	9	3	28
	56	31	27	13	7	44	27	12	10	50
	Totals	98	130	63	39	73	47	30	17	35
BC_5	57	36	31	10	6	22	20	3	7	43
	58	52	52	19	10	33	2	5	1	18
	59	36	35	19	7	20	8	16	2	32
	60	27	29	6	5	33	35	4	9	55
	Totals	151	147	54	28	108	65	28	19	36
BC_6	61	5	15	2	4	10	9	6	5	55
	62	23	31	10	16	23	15	18	7	44
	63	28	22	4	3	37	18	11	4	55
	64	10	15	2	3	36	18	6	7	70
	65	69	56	25	13	17	11	5	7	19
	66	16	10	4	2	27	4	1	1	56
	67	27	30	8	7	70	35	16	16	63
	Totals	178	179	55	48	220	110	63	47	48
BC_7	68	10	32	10	4	26	45	10	7	61
	69	16	21	7	4	24	34	8	8	60
	70	26	39	12	7	42	36	21	19	58
	Totals	52	92	29	15	92	115	39	34	60
Grand totals		1738	2068	738	495	918	510	270	172	27

for some of those in chromosomes II and III, which involved crossing over. In culture 19, 70 percent of the tumors were with star, showing that the star chromosome had received by crossing over a very effective tumor gene.

DICHAETE HAIRLESS × TUMOR, FEMALE BACKCROSS

The backcross of tumor to star and dichaete had showed that there was a main gene for tumor located in the third chromosome roughly ten units distant from dichaete. To determine this distance more accurately and also to find whether the locus of the tumor gene was to the left of dichaete at roughly 30 or to the right at roughly 50, a backcross was made involving tumor and the dominant hairless in addition to dichaete. The locus of hairless is about 30 units to the right of dichaete (that is, at 69.5).

In the backcross results (cultures 37 to 41, table 4) only one tumor dichaete fly occurred, while 23 tumor dichaete hairless flies occurred. The relative difficulty in the formation of the tumor dichaete class indicated that it was the class due to double crossing over, and hence, the locus of tumor was to the left of dichaete and still farther to the left of hairless. The percentage of recombination for tumor and dichaete, figured on the basis of the tumor-bearing flies, was 17, a value somewhat higher than the 10 found in the star dichaete × tumor backcross. In the *D H* × t_u results only 8 percent of the flies bore tumors, and that again showed that tumor was a polygenic character.

Backcrosses were continued for several generations by crossing *D H* females from one of the cultures giving the highest percentages of tumors, to tumor brothers (table 4). In each successive generation the average percentage of tumors rose, reaching 60 in the seventh backcross generation (BC_7). The various frequencies of tumors represent the various combinations of tumor genes, with increasing homozygosis.

In table 5 the cultures have been collected according to the percentage of tumors shown. A comparison of the groups shows an increasing percentage frequency of tumors with dichaete, showing that certain of the tumor genes that have become homozygous were in the third chromosome. But the increase in tumors with dichaete was not as rapid as the general increase in tumor frequency, which indicated that genes outside chromosome III were likewise becoming homozygous. The recombination for dichaete and hairless did not vary greatly, and the average percentage for the grand total was 24.3, which was close to the standard value of 25.7.

TABLE 5

Cultures of table 4 grouped according to percentage of tumors.

GROUP	*D H*	+	*D*	*H*	*tu*	*tu D H*	*tu H*	*tu D*	MEAN PERCENT	*tu-D* RECOMBINATION	*D-H* RECOMBINATION
0–10	596	801	225	194	90	20	15	2	7	17	22
10–20	539	620	227	143	177	63	45	25	17	28	24
20–35	241	227	133	65	151	54	51	18	29	25	28
35–55	197	207	98	54	195	139	76	51	45	41	27
55–70	165	213	55	39	305	234	83	76	60	44	22
Total	1738	2068	738	495	918	510	270	172	27	36	24.3

ROUGHOID TUMOR×DICHAETE, FEMALE BACKCROSS

The dichaete hairless×tumor backcross had given the locus of the main third-chromosome tumor gene at about 17 units to the left of dichaete. The value 17 was based on rather small numbers, and was in disagreement with the value 10 found in the star dichaete×tumor backcross. The locus of tumor should, in any case, be rather close to that of hairy, which is about 15 units to the left of dichaete. But the uncertainty in the order of loci, and the closeness of the loci together would make it very difficult to obtain a double recessive of hairy with tumor. Instead, the locus of tumor was checked by the use of roughoid, which lies at the zero of the third chromosome. A tumor-bearing female was mated to a male that was roughoid hairy scarlet peach spineless sooty, and the F_1 wild-type flies were inbred. From the F_2 flies those were selected that showed roughoid but none of the other third-chromosome characters. Such flies resulted from crossing over between roughoid and hairy, and should carry with roughoid all of the tumor chromosome that lay to the right of the point of crossing over. When the F_2 roughoid flies were inbred, a few flies were found in F_3 that showed both roughoid and tumor. Some of these were bred together, giving a roughoid tumor stock, which, however, threw only about 15 percent of tumors. Dichaete females were crossed to roughoid tumor males, and the F_1 dichaete females were crossed to roughoid tumor males from the stock (upper division of table 6). In the backcross only a very few tumor flies appeared, about 2 percent of the total (totals by generations given in table 7). Such a low value was in conformity with the known polygenic nature of the tumor character, and this value was lower than that in the dichaete hairless×tumor backcross, because the roughoid tumor stock used was one that did not contain the full complement of tumor genes. The few tumor flies that did appear in the

backcross were distributed nearly at random with respect to roughoid and dichaete, and no conclusions as to order and distance could be safely drawn.

TABLE 6

P_1, dichaete ♀ × roughoid tumor ♂; backcross F_1D ♀ × ru t_u ♂.

GENERATION	CULTURE NUMBER		D	D r_u	+	r_u	r_u t_u	t_u	r_u t_u D	$_u$ D	PERCENT t_u
BC		71	50	19	36	18	..	1	1	..	2
		72	84	20	30	24	..	1	..	2	2
		73	56	17	51	38	..	1	..	..	1
		74	44	21	35	40	2	..	..	1	2
		75	88	18	98	7	3	1	..	1	2
		76	46	41	47	57	1	..	1	1	2
BC_2	71	77	146	80	90	133	12	2	..	..	3
		78	102	46	80	113	1	2	..	1	1
	72	79	98	60	78	90	1	..	..	..	0
		80	48	33	32	34	..	..	..	2	1
		81	122	64	81	95	1	2	..	1	1
		82	82	45	32	43	17	1	2	..	9
		83	72	18	74	28	..	..	..	1	1
	74	84	66	30	26	36	14	7	5	..	14
		85	86	64	44	65	37	13	4	2	18
		86	94	46	51	74	12	10	3	1	9
		87	116	37	28	87	36	12	2	2	16
		88	85	49	65	73	25	5	4	1	11
		89	44	22	35	34	10	7	4	1	14
	76	90	75	66	72	70	7	6	..	..	4
BC_3	77	91	192	98	96	128	56	16	9	..	14
	82	92	94	56	45	27	52	27	12	2	30
		93	130	93	76	64	80	21	18	2	25
		94	145	76	79	83	42	22	7	3	16
		95	133	92	97	88	53	20	7	..	16
	84	96	110	67	71	84	38	15	9	3	16
	85	97	234	113	128	120	61	33	10	5	15
		98	31	12	21	14	35	6	6	2	39
	86	99	239	103	141	161	73	33	22	4	17
BC_4	93	100	35	15	25	14	22	12	4	..	30
	96	101	15	17	15	8	28	10	1	1	42
		102	122	52	45	26	108	43	17	4	41
		103	75	38	27	24	42	27	13	2	34
		104	70	29	28	12	63	25	16	5	44
		105	89	44	48	16	86	33	40	2	45
	97	106	129	80	72	81	32	21	10	1	15
	99	107	90	56	57	24	70	27	17	3	34
		108	19	15	7	10	23	9	1	1	40

TABLE 6 (continued)

GENERATION	CULTURE NUMBER		D	D ryu	+	ru	ru lu	lu	ru lu D	ru D	PERCENT lu
BC_3	102	109	163	66	50	33	148	59	29	9	44
		110	95	30	43	36	94	21	12	4	38
		111	72	29	18	17	50	16	16	4	39
	107	112	151	79	68	52	100	43	23	7	33
		113	156	88	76	68	100	42	28	3	31
BC_4	109	114	201	93	60	37	182	83	38	7	44
		115	64	28	34	10	62	38	16	6	47
		116	124	58	54	10	123	50	33	5	46
		117	149	83	50	43	84	34	19	4	30
	112	118	137	87	68	53	111	54	31	16	38
	113	119	39	15	28	4	53	31	9	2	52
		120	44	30	28	6	53	15	9	..	42
		121	28	14	9	6	22	8	8	..	40

TABLE 7

Cultures of table 6 totaled according to generations.

GENERATION	D	D ru	+	ru	ru lu	lu	ru lu D	lu D	PERCENT lu	ru-u	lu-D	ru-D
BC	368	136	297	184	6	4	2	5	2	...	..	33
BC_2	1246	660	788	975	173	67	24	12	7	29	13	39
BC_3	1308	710	754	769	490	193	100	21	18	27	15	40
BC_4	644	346	324	215	474	207	119	19	35	28	17	42
BC_5	637	292	255	206	492	181	108	27	37	26	17	38
BC_6	786	408	331	169	690	313	163	40	42	28	17	42
Totals	4979	2552	2749	2518	2325	965	516	124	24	27.7	16.3	40.5

From some of the backcross cultures dichaete females and roughoid tumor males were mated for another generation (BC_2). In this generation about half of the cultures gave the same low percentages of tumors as occurred in the first backcross, but the others gave from 9 to 18 percent of tumors. These cultures evidently represent a new level of tumor production due to the combination in the parents of more tumor genes than were together in the original backcross. The distribution of tumors with respect to roughoid and dichaete became significant with the lowest class, tumor dichaete—that due to double crossing over. The order of loci was thus roughoid, tumor, dichaete, as expected from the results with dichaete hairless.

Four more generations were raised, using parents from the cultures in which the best combinations of tumor genes were present. The percentage

of tumors rose by steps, but the r_u-t_u and the t_u-D recombination percentages remained nearly constant, with averages for the whole data of 27.7 and 16.3. The sum of these two values is 44, which is 3.6 points higher than the standard r_u-D crossing over total of 40.4. Evidently, crossing over was a little higher in these stocks than in standard stocks. If the locus of tumor is calculated as to its proportionate location on a standard map, it is found to be 15 units to the left of dichaete, or at 25.4.

The cultures of table 6 were plotted according to the percentage of tumors, and they were found to fall into four groups. Thirteen cultures showed percentages below 5, mostly with 2 percent of tumors. Then, between 5 and 9 there were no cultures. A second group had a mean at 15, a mode at 16, and a range from 9 to 19. An isolated culture at 25 has been included with the third group, whose mean was at 31, and range, outside the one at 25, from 30 to 35. The fourth group had a mean at 43 and a range from 38 to 47 with an extreme culture at 52. The cultures in each of the four groups were totaled (table 8) and the percentages of recombination calculated. In the group whose range was from 0 to 5, the incidence of tumor was very irregular, suggesting that with so low a genetic basis for tumor production, environmental factors were relatively more effective in influencing the result. In the remaining three groups the recombination percentages were constant, showing that the rise in percentage of tumors was mainly due to the accumulation of tumor genes in other chromosomes than the third, and possibly in the third but in the region to the right of dichaete.

TABLE 8

Cultures of table 6 grouped according to percentage of tumors.

GROUP	D	$D\ r_u$	+	r_u	$r_u\ t_u$	t_u	$r^u\ t_u\ D$	$t_u\ D$	PERCENT t_u	r_u-t_u	t_u-D	r_u-D
0-5	1031	503	804	747	28	16	2	10	2	46	21	42
9-19	1765	922	965	1154	506	215	98	23	15	28	14	41
25-35	880	508	424	316	550	233	134	23	31	27	17	42
38-53	1313	619	556	298	1241	501	282	68	43	27	17	40

TUMOR × BLACK PURPLE

The star dichaete × tumor backcross had shown that there was free recombination of star and tumor, and therefore, that the locus of the principal second-chromosome tumor gene was probably in the region near, or to the right of, black. Since there were at that time no usable dominant characters in the region to the right of black the location must

be determined by using two recessives. According to the star tumor recombination percent the tumor gene might lie rather near black, and so black and purple seemed as far to the right as would be safe to use. To secure a stock of black purple with tumor, tumor females were mated to black purple vestigial males. F_2 flies that were black and purple but were not-vestigial were mated together. Such flies had the right end of the chromosome replaced, through crossing over, by the corresponding right end of the second chromosome of the tumor stock. In F_3, a few black purple tumor flies appeared, which were inbred to give a stock.

In the roughoid tumor×dichaete backcross the percentage of tumors in the first backcross generation had been very low and the distribution of tumors not significant. This meant that the data were nearly useless in the problem of finding the locus of the gene. In subsequent generations more tumor genes outside the region under test were brought together and the data of these later generations were satisfactory. In order to secure, in the first place, a favorable combination of tumor genes outside the region under test, tumor flies were mated to vestigial females. The vestigial flies that appeared in F_2 might have first-, third- and fourth-chromosome tumor genes, but were prevented from having second-chromosome genes by the selection of vestigial, which marks the non-tumor second chromosome. Crossing over might introduce into the vestigial chromosome tumor genes to the right or to the left of vestigial, but the star×tumor backcross had shown that there were probably no very important genes to the left of black, and not a great proportion of the vestigials would be crossovers to the right of vestigial. By starting from several F_2 vestigials, the type of result given by the majority of lines would be that in which crossing over had not occurred. The "B C" cultures of table 9 were from four such lines; and since they were roughly similar, it is probable that they were all of the non-crossover type. The percentage of tumors was high even in the first backcross generation. The recombination of tumor with black and purple was very free, as shown by the large number of not-black not-purple tumor flies and also by the numerous black purple not-tumor flies. Hence, the locus of the tumor gene must have been very far to the right of black and purple, probably close to speck in the extreme right end. No close calculation of the position could be made from the data, since it became apparent that the difficulty of detecting tumors in black flies was much greater than in not-black flies. This fact explained the *b* p_r class being seemingly greater than the *b* p_r t_u class, a high proportion of the flies that were actually tumor-bearing were classified as non-tumored. Two subsequent back-

TABLE 9

P_1, wild ♀ × black purple tumor ♂; backcross, F_1 + ♀ × b p_r t_u ♂.

GENERATION	CULTURE NUMBER	*b* p_r	*b* p_r t_u	+	t_u	*b*^{1}	*b* t_u	p_r	p_r t_u	PERCENT t^u
BC	200	77	73	57	77	1	..	1	1	53
	201	62	20	21	31	2	..	5	2	37
	202	31	35	31	29	2	..	3	1	49
	203	97	13	90	18	..	..	2	..	14
	204	28	52	29	41	..	..	1	1	62
	205	51	32	58	48	1	..	1	1	42
	206	34	22	33	32	3	..	12	3	41
	207	33	15	41	35	..	..	..	2	45
	208	101	42	63	31	1	..	1	..	27
	209	12	13	9	11	..	..	..	..	53
	Totals	526	317	432	353	10	0	26	11	41
BC_2	204 210	16	50	24	60	1	..	1	3	73
	204 211	99	88	82	104	..	1	4	3	51
	204 212	112	62	114	91	5	5	4	8	41
	207 213	48	18	65	37	1	4	..	1	34
	200 214	91	71	47	106	2	5	6	1	56
	200 215	119	93	54	151	3	9	2	2	59
	200 216	158	89	87	98	3	3	..	..	43
	200 217	161	66	119	138	2	1	3	5	42
	218	80	85	128	129	..	..	1	..	51
	219	80	60	77	86	2	2	1	..	48
	206 220	179	50	181	55	2	3	6	..	23
	206 221	85	64	64	103	1	1	1	..	53
	209 222	57	47	34	50	1	..	..	..	51
	209 223	75	79	38	105	1	3	3	13	63
	209 224	121	61	62	108	2	..	1	2	48
	Totals	1481	983	1176	1421	26	37	33	38	48
BC_3	214 225	79	21	48	62	3	..	1	..	39
	214 226	130	39	84	109	10	4	4	1	40
	214 227	73	64	76	85	..	7	2	2	51
	211 228	69	50	53	57	1	3	2	..	47
	211 229	102	54	96	68	..	8	2	1	40
	211 230	86	30	77	37	..	2	..	..	30
	213 231	82	31	46	61	..	1	3	3	42
	213 232	68	38	55	83	..	2	1	..	50
	220 233	68	26	70	52	2	..	3	1	36
	220 234	74	58	87	66	9	2	1	4	43
	215 236	44	12	31	20	..	1	..	..	28
	215 237	57	60	34	89	6	1	1	4	61
	215 238	17	30	9	31	1	1	..	..	70
	Totals	949	513	766	820	32	32	20	16	44
	Grand totals	2956	1813	2374	2594	68	69	79	65	45

TABLE 10

P_1, bar ♀ × tumor ♂; backcross, F_1B ♀ × t_{tu} ♂.

CULTURE NUMBER	+	t_{tu}	B	t_{tu} B	PERCENT t_{tu}
249	142	30	138	20	15
250	117	36	197	44	20
251	343	47	311	47	13
252	182	19	165	24	11
253	262	18	200	14	6
254	270	55	249	40	15
255	116	29	115	31	21
Totals	1432	234	1375	220	14

cross generations showed no new relation. For the whole data the black-purple recombination percent was 2.8, instead of the standard 6. A similar reduction, to about 3, had been met with in other experiments, and was due to a specific crossing over reducer. The presence of a crossing over reducer made little difference, for the only conclusion that could be drawn from the data was that the locus of the principal second-chromosome tumor gene was probably rather near the right end of the chromosome.

BAR × TUMOR

The star dichaete × tumor backcross had shown that roughly a fifth of the flies of a tumor stock were made tumored by the added presence of genes outside the second and third chromosomes. The test for the presence in various parts of the X, of tumor genes of such slight effect, would be difficult. Only one experiment seemed simple enough to be worth a trial. The dominant mutant bar, with a locus at 57, might show non-random recombination with tumor, provided the locus of the tumor gene was rather close to that of bar. The bar × tumor backcross (table 10) showed no difference in the distribution of tumors to the bar and to the not-bar flies, 14 percent of the bar flies bore tumors and likewise 14 percent of the not-bars were tumor-bearing. Either the genes responsible for the above-mentioned margin were in the fourth chromosome rather than the X, or they were rather far to the left of bar in the X.

EYELESS × TUMOR

To test for the presence of fourth-chromosome tumor genes a tumor-bearing male was crossed to the fourth-chromosome recessive eyeless; and several cultures of F_2 were raised (table 11). Only 2 percent of the F_2 flies were tumor-bearing. The percentage of tumors in the eyeless

TABLE 11

P_1, eyeless ♀ × tumor ♂; F_2, F_1 + ♀ × F_1 + ♂.

CULTURE NUMBERS	+	*t_u*	*ey*	*ey t_u*	PERCENT *t_u*
256	450	12	81	1	2
257	329	12	59	4	4
258	333	7	57	1	2
259	222	1	60	..	..
260	204	4	37	1	2
261	535	5	69	1	1
262	351	4	58	..	1
263	495	8	78	..	1
264	308	9	59	..	2
Totals	3227	63	558	8	2

TABLE 12

P_1, tumor ♀ × eyeless, ♂; backcross, t_u e_y ♀ × F_1 + ♂

CULTURE NUMBERS	+	*t_u*	*ey*	*ey t_u*	PERCENT *t_u*
265	136	41	77	13	20
266	112	36	93	30	24
267	175	48	133	25	19
268	202	37	100	15	15
269	142	23	47	5	13
270	35	13	23	16	33
271	169	31	101	18	15
Totals	971	229	671	122	18

was slightly, but not significantly, less than in the not-eyeless flies. Some of the eyeless tumor flies were inbred, and about 45 percent of the offspring bore tumors. A P_1 mating of eyeless to tumor gave F_1 males and these were backcrossed to eyeless tumor females of the above stock (table 12). About 18 percent of the backcross flies bore tumors, and of these the percentage of eyeless (15) was lower than the percentage in the not-eyeless (19). This difference was not great, but was as great as would be expected if there were present a fourth-chromosome modifier of the degree of effectiveness indicated by the results of the previous crosses.

SUMMARY

The development of the tumors studied was found to depend upon the action of differential genes that were numerous and that differed in effectiveness. The gene having greatest effect was a third-chromosome reces-

sive located about 25 units to the right of roughoid and 15 to the left of dichaete. The second most effective gene was located in the second chromosome in the neighborhood of speck at the right end. About 80 percent of the total effect was due to genes in second and third chromosomes, and 20 percent to genes in the first and fourth.

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MATURATION IN DIPLOID AND TRIPLOID TOMATOES[1]

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INTRODUCTION

In the past few years numerous observations have been made which seem to indicate that abnormalities in chromosome distribution are less uncommon than was formerly thought. Triploidy appears to be one of the more frequent types, having been described for mosses, Morus, Canna, Datura, Hyacinthus, Uvularia, Oenothera, and Drosophila. Up to the present time no complete study of the early meiotic prophase of a triploid has been published although several authors have reported on the chromosome distribution and a few have studied the forms of the trisomes at diakinesis. It was thought that such an investigation might be useful both as a means of helping in the clarification of the vexed question of parasynapsis *versus* telosynapsis, and of determining the extent to which crossing over may be expected between members of a trisomic set of chromosomes in the tomato.

MATERIAL AND METHOD

Roots, anthers and ovaries from a triploid plant of *Solanum lycopersicum* L. were fixed in chrom-acetic-urea and stained with Heidenhain's

[1] Contribution from the Genetics Division of the UNIVERSITY OF CALIFORNIA.

iron haematoxylin. Anthers were also fixed in Flemming's strong. A short description of the plant and its origin has already been published (LESLEY and MANN 1925). Chromosome counts from pollen mother cells were made from smears stained by Belling's aceto-carmine method. Sections of whole buds were made to determine the time of maturation in the ovules in relation to that in the pollen mother cells. It was found in general that ovaries contained the desired stages when the pollen mother cells were in cytokinesis or immediately thereafter. Sometimes different anthers of the same flower were in very different stages and many degenerating anthers were found in both diploid and triploid buds. The genetics of the triploid plant is being investigated by J. W. LESLEY and it is to him that I owe the privilege of working on this material.

MATURATION IN THE DIPLOID

Seriation of stages

The maturation processes in the tomato were first studied from megaspore mother cells but since ovarian material does not give any adequate clue to seriation of certain critical stages, longitudinal sections of anthers were used for comparison. As long ago as 1904 BERGHS stated that neighboring cells in the anther sac of *Lilium lancifolium* often show a graded series of stages which give one a clue to the natural seriation in meiosis. JANSSENS (1905) speaking of the natural seriation shown by the testis of Batrachoceps says (p. 380)

> "We find ourselves then in the presence of an organ, which from the point of view of seriation of stages, offers the same advantage as the locules of the anthers of the Liliaceae."

No one anther provides a complete series in tomato, but a study of numerous anthers gives one an overlapping set of series from which most of the desired facts may be ascertained. The youngest stages are found at the proximal end; the oldest may be either at the distal end or near the center of the locule, usually the former. While the two locules of one anther contain approximately the same stages of meiosis, one may be slightly in advance of the other. The stages in the different anthers have been arranged in their natural order of progression from presynapsis to cytokinesis and are given below:

(1) Presynapsis (usually occupies whole locule)
(2) Leptotene to synezesis
(3) Synezesis to pachytene
(4) Synezesis (few cells at base of anther) pachytene to first anaphase
(5) Diakinesis to first telophase or sometimes second anaphase

(6) First metaphase to second anaphase
(7) Second anaphase to cytokinesis
(8) Cytokinesis and young pollen cells

Nuclear changes during meiosis

In the tomato one finds a true spireme (figure 1) and typical parasynapsis (figure 2), the stages and their sequence conforming very accurately to those described by BERGHS (1904). These descriptions differ from the typical parasynaptic scheme as presented by SHARP (1921, p. 231, scheme A, figure 83) in that there is a continuous spireme and that following the pachynema stage (figure 3) the threads lose some of their affinity for stain and again appear as thin paired threads which then shorten, thicken, and become separated as the pairs of diakinesis (figures 4 to 8). One finds the usual rings, V's, and laterally paired rods at diakinesis, consisting of homologues variously attached to one another (figure 8). The changes observed in the tomato following pachynema are of the same type as those which occur in animal oögenesis, but they are less extreme. The same stages were found in both ♀ and ♂ sporogenesis. No figures of synezesis have been presented, because, as usual, it is difficult to see much at its height. It was noted, however, that longitudinal pairing of threads precedes synezesis, that they remain paired during it, and emerge in the same condition. The chromosome number of the normal diploid tomato is N = 12, as WINKLER (1910) stated. Twelve pairs and regular meiotic divisions are certainly the usual thing. The chromosomes are short rods showing no distinct individuality.

Orientation of spindles in anther loculi

It was noted with great interest that the pollen mother cells are not oriented at random in the anther loculi at and immediately following the first meiotic division, but that a longitudinal section at right angles to the septum between loculi shows only lateral views of spindles. As one would expect, therefore, cross sections show many polar views of first-metaphase and anaphase groups. The second-metaphase figures are oriented at random.

The cytoplasm during meiosis

During meiosis the cytoplasm shows certain typical changes which are probably the result of the division phenomena. At and before diakinesis it appears to be of fairly uniform and densely granular structure. Just after the disappearance of the nuclear wall, however, one notes a denser area about the chromosome group at about the place which the nuclear

LEGEND FOR PLATE 1

FIGURES 1 to 7.—Nuclear changes in successive stages in the prophases of meiosis in the embryo mother cell of the diploid tomato.

FIGURE 8.—Typical diakinesis chromosomes in the diploid.

1
2
3
4
5
6
a
b
7
a
b
8

wall previously occupied, while the region between this and the chromosomes is practically free of granules and that between the granular area and the cell wall is slightly less opaque. WINKLER (1916) shows this cytoplasmic differentiation in his figures of first-metaphase plates. During the development of the first-metaphase plate and in early anaphase this sphere becomes unequal in thickness, usually appearing as a wedge-shaped mass, now very distinctly denser than the rest of the protoplasm and taking a blue stain in haematoxylin. In early telephase, just before and while the nuclei are enlarging, the cytoplasm again appears almost uniformly granular, although the region immediately surrounding the barrel-shaped remnant of the spindle is slightly denser than the rest of the cytoplasm. As the nuclei enlarge this denser region is compressed to form a spool-shaped body which stains blue, and after the nuclear walls disappear this remains, the rest of the cytoplasm being almost granule-free (clear white after fixation in chrom-acetic-urea). From this time until early second telophase this densely granular structure usually retains its form, but in a few cases when the two spindles lie in slightly different planes and at a slight angle to each other, it has been seen as two crescent-shaped halves each of which conforms to one side of a spindle. As the nuclei of the second telophase begin to form and the spindles are rotating to their final position before cytokinesis (such as to give the usual tetrahedral arrangement of pollen cells) the cytoplasm again becomes fairly uniform in density. The chief interest in the process described above lies in its bearing on the times and to some extent the method of cytoplasmic movements during the nuclear division. The spindle is obviously a very effective barrier to lateral transfer of cytoplasmic granules and when two spindles are present each forms a center of activity which affects the intervening region very slightly if at all. At the telophase, as the nuclei take in fluid, general cytoplasmic movements occur, resulting in an even distribution of granules. This is probably facilitated at the second division by the movement of the spindles. Somewhat similar changes in the cytoplasm are seen during mitosis in root-tip cells.

Number of archesporial cells

In the diploid tomato one occasionally finds a several-celled archesporium, and in a very few cases two megaspore mother cells were seen in meiosis. A partially sterile double trisomic plant (26 chromosomes) showed this condition very frequently, occasionally as many as three cells undergoing reduction in one ovule. Such twin and triplet megaspore

LEGEND FOR PLATE 2

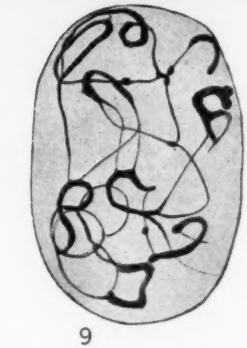
FIGURES 9 to 12.—Nuclear changes in the prophase of meiosis in the triploid tomato, showing alternate double and single threads.

FIGURE 13.—A pre-diakinesis stage from a pollen mother cell of the triploid which shows the alternate single and double threads especially clearly. Note that the double portions of the trisomes are comparable to the paired chromosomes of the diploid (figure 7).

FIGURES 14a and 14b show most of the 12 trisomes in early diakinesis of an embryo-sac mother cell.

FIGURE 15 a to d shows four slightly different trisome formations from a pollen mother cell.

FIGURE 16.—A typical somatic metaphase of the triploid.



9

11

10

12

13

14 a

14 b

a b c d

15

16

mother cells may or may not mature synchronously. In one case one megaspore mother cell was in diakinesis, while its mate was in first metaphase. COULTER and CHAMBERLAIN (1912, p. 62) give evidence which shows that a many-celled archesporium is sometimes found in a number of the Sympetalae. The triploid, like the diploid, shows two megaspore mother cells only rarely. Two megaspore mother cells in synapsis are shown in text figure 1.

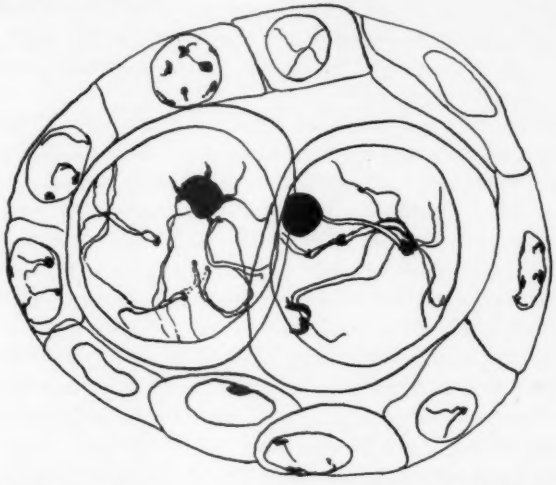


FIGURE 1.—Two embryo-sac mother cells undergoing reduction within the same megasporangium.

MATURATION IN THE TRIPLOID

Nuclear changes during meiosis and assortment of chromosomes

As in the diploid tomato the seriation seen in longitudinal sections of anthers was of great service. At the earliest stages the nucleus contains unpaired threads, most, at least, of which are united to form a spireme. One next finds a spireme which is double parasynaptically in some parts and single in others (plate 2, figures 9, 10, 11). As this shortens, the doubled regions of the spireme may remain in contact throughout their length, or they may only remain attached at the tips of the chromosomes (plate 2, figures 10, 11 and 12). In diakinesis trisomes most frequently consist of two chromosomes attached at both tips to form a ring, the third chromosome being united to them at one of these points of union (plate 2, figures 14a and 15a). They also appear as a pair laterally united

with the third element joined to the tip of one member of the pair (figures 14a and 14b), or as three chromosomes joined end to end (figures 15c and 15d). The latter type results from the opening out of the paired chromosomes to form a V (figure 15, b, c and d). It was noted above that the chromosomes of the diploid form rings, parallel rods, or V's consisting of homologues joined end to end so that the configurations seen in the triploid differ only in the presence of the unpaired element which bears a telosynaptic relation to the trisomic group. This third element often shortens more slowly than the pair and seems not to be as firmly attached to it as they are to each other, since a few univalents are not uncommonly seen at the late prophase.

When this study was begun it was thought that one could differentiate in a triploid between splitting and parasynaptic union of chromosome threads, because it seemed possible that the three threads might all unite parasynaptically. This does not occur, but the alternation of paired and unpaired threads in the spireme together with their relationship to the chromosomes in diakinesis is quite as convincing evidence that the two threads seen in the diploid are parasynaptically united and are not the result of longitudinal division of the spireme. It is also of interest that the third element is joined telosynaptically to the other two chromosomes. One usually finds 12 trisomes at diakinesis, but at a later stage, in the first metaphase, one to several single chromosomes are sometimes seen. At this time the trisomes look like three balls of the same size. The univalents are easily distinguished from the bivalents or trisomes. Four megaspores may be formed, or one of the first two cells may degenerate without further division. Apparently some embryo formation occurs in the triploid, but the material was not well enough fixed to show anything about the proportion of embryos that begin to develop. It is known (LESLEY and MANN 1925) that the triploid has some viable ovules, since a few seeds result from fertilization with diploid pollen. The pollen of the triploid has thus far proven useless either on diploid or triploid plants. From the cytological observations one would expect a high percentage of sterility, but a study of meiosis does not show why it should be as bad as the experimental results demonstrate it to be. The following chromosome counts taken from 94 second-metaphase figures from pollen mother cells show that the chromosomes assort at random:

TABLE 1

Distribution of pollen mother cells with respect to number of chromosomes

Number of chromosomes	12	13	14	15	16	17	18	19	20	21	22	23	24
Frequency	1	1	6	4	10	23	22	13	4	7	0	2	1

Lagging chromosomes are sometimes seen, and these very occasionally divide or are left in the spindle where they form micronuclei, but the divisions are usually clean. Cytokinesis gives rise to four to eight pollen cells, usually four, which sometimes differ in size. Occasionally one pollen cell divides, forming a little tetrad.

A longitudinal section of an ovary of the triploid in which the megaspore mother cells were mostly in synapsis had a sac on one side of the style in which a mass of microspore mother cells were at the same stage, while the normal anthers of the same flower contained pollen mother cells in early cytokinesis.

LITERATURE AND DISCUSSION

The term triploid has been used rather loosely to designate plants which have originated by hybridization between species, one of which has twice the chromosome number of the other, as well as for forms in which the triploid number of chromosomes has resulted from the doubling of one set of chromosomes within a species. This fact should be kept in mind in any discussion of triploidy, because the two conditions need not be entirely analogous. The discussion which follows will consider only cases of the latter type, to which the tomato triploid belongs.

Where the origin of triploids has been observed, they have either appeared suddenly as mutations, as one or a few plants in a diploid population, or they have been produced secondarily by crossing a diploid with a tetraploid which has so originated. The tomato triploid (LESLEY and MANN 1925) appeared as one unfruitful, somewhat gigantic plant in a plot of 90 normal ones. We do not yet know much about the means by which triploids originate, but they probably depend upon the origin either of tetraploid cells or of a tetraploid plant with subsequent fertilization of a tetraploid by a diploid germ cell. WINKLER (1916) reports the occurrence of a sterile tetraploid tomato following grafting of *S. lycopersicum* tissues on nightshade (*S. nigrum*), but no tetraploid plants have thus far been observed in Mr. LESLEY's cultures. However, roots of diploid plants do sometimes contain large tetraploid portions (M. M. LESLEY 1925), and it would therefore not be impossible that occasional tetraploid gametes might be present. These plants have not yet produced tetraploid shoots nor have they been examined for occasional tetraploid germ cells, so that thus far the production of triploid plants is our only evidence that tetraploid germ cells occur. The sterility of WINKLER's tetraploid plant is somewhat surprising since triploid plants are found to occur. Several tetraploid branches have been found on diploid Datura plants and these

have produced tetraploid offspring (BLAKESLEE and BELLING 1924). L. V. MORGAN (1925) describes the occurrence of a tetraploid female Drosophila among the progeny of a triploid female. It was noted above that the chromosomes of the triploid tomato appear in diakinesis as twelve groups of three each, or as trisomes. This has also been found in the mosses and in Canna, Morus, Datura, Hyacinthus and Drosophila, but in the Oenothera triploids GEERTS (1911) found 7 pairs and 7 unpaired chromosomes, while GATES (1909) found no pairing or very loose pairing in the triploids, and a similar condition in the tetraploid (GATES 1911). METZ (1922) described parasynaptic union of the four homologous threads in Sarcophaga at diakinesis, but the figures given by WINKLER (1916) for the heterotypic metaphase of his tetraploid tomato show no tendency towards grouping into quadrivalents. The trisomes of Drosophila (METZ 1923) differ from those of Datura and tomato in that the former appear side by side in threes at the heterotypic metaphase, whereas the chromosomes of the trivalents of the two latter commonly remain attached by the tips only. The trisomes found in tomato are very similar to those described for Datura (BELLING and BLAKESLEE 1923). BLAKESLEE (in DAVENPORT 1924, p. 28) says,

> "It has been shown that in haploid, diploid, triploid, and tetraploid Daturas, the chromsomes at the metaphase are joined in such a way that the homologous chromosomoes are in contact at the ends only. (This is not the case in some other plants such as Hyacinthus and Uvularia.) In the above mentioned Daturas the chromosomes are grouped in configurations which agree with the assumption that only corresponding or homologous ends come together".

The early prophase has not yet been described for the Datura triploids, but the similarity of the diakinesis stages in Datura and tomato indicate that it is possible that the earlier stages are also alike in these two Solanaceous plants. It appears, then, that all three chromosomes of a trisomic set probably pair side by side or parasynaptically in Drosophila, while in the tomato and possibly in Datura two of them unite parasynaptically, the third being attached either to the tip of one member of the pair or between their tips. The diploid tomato shows true parasynapsis, but in the triploid a parasynaptic union between all three chromosomes occurs rarely if ever.

The Diptera are outstanding in that even in somatic metaphase, homologues lie side by side. This association is even closer in the somatic prophase (METZ 1922). The somatic prophase of the tomato has not yet been carefully studied, but the homologous chromosomes seem, like those of most plants, to show no such "attraction" in the metaphase.

Since synapsis is different in tomato and in Drosophila one would expect breeding results to differ in that crossing over between three sets of genes would be expected in Drosophila, but not in the triploid tomato. There is as yet no evidence on this point from the tomato, but crossing over between all the X chromosomes sometimes occurs in the triploid of Drosophila (MORGAN, STURTEVANT and BRIDGES 1924; L. V. MORGAN 1925).

The trisomes of Datura and tomato resemble the figures which show an X chromosome attached to an autosome (WILSON 1925, p. 776, figure 372). In diakinesis twelve trisomes are found in Datura and tomato, but at the metaphase each may show one to several univalents and a corresponding number of bivalents. The first division is far more regular in appearance than is usual in "triploid" species hybrids, in which, as for example in the 7×14 wheat species hybrid (KIHARA 1919), there are typically 7 pairs and 7 univalents at the first metaphase. The lagging of univalent chromosomes appears to be due to the fact that it takes longer for chromosomes to divide equationally than for them to assort. This difficulty is lessened for most of the chromosomes of the triploid by their trivalent condition. The lagging chromosomes of the first division are restricted to those few that fail to remain attached to their mates. The two chromosomes which have paired parasynaptically tend to remain together, the telosynaptically attached one tending to disjoin. This is suggestive of the observations on telosynapsis in Oenothera, in which pairing is sometimes so weak that it is difficult to distinguish chromosome pairs at the metaphase (DAVIS 1911). Observations on distribution of chromosomes in triploid pollen mother cells indicate that independent assortment of double or single chromosomes is the rule, at least in Datura, Hyacinthus and the tomato. The agreement of numbers in the different chromosome-number classes at the second metaphase, with the expectation as well as observations of lateral first anaphase, show that division of univalents is rare at the first division. As in Hyacinthus (BELLING 1924), the slightly greater number of counts in the lower number classes in the tomato is doubtless due to the greater ease in finding accurately countable figures with low than with high numbers. More laggards are to be seen in the second than in the first division in the tomato.

GATES (1925) states that triploids can perpetuate themselves only by apogamy or by becoming hexaploid. The triploid tomato is not apogamous, so that it can only be maintained by cuttings.

SUMMARY AND CONCLUSIONS

1. In the diploid tomato there is a true spireme and typical parasynapsis.

2. In the triploid the spireme consists, after synapsis, of a series of alternating paired and unpaired threads. The paired threads correspond to the parasynaptically paired strands of the diploid. No parasynaptic union between three threads was observed.

3. On the basis of the observations on synapsis in the triploid tomato one would not expect to find crossing over between all of these strands, although presumably it might occur between any two chromosomes.

4. At diakinesis there are usually twelve trisomes similar to those found in Datura, but univalent chromosomes are sometimes seen.

5. The chromosomes assort independently at the first meiotic division.

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CRIPPLED, A NEW MUTANT CHARACTER OF *DROSOPHILA MELANOGASTER*, AND ITS INHERITANCE

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INTRODUCTION

This work was done in the Zoology Department of COLUMBIA UNIVERSITY from September 1923 to June 1924 and in the MARINE BIOLOGICAL LABORATORY, Woods Hole, from June to September 1924. I am very grateful for the hospitality shown to me by the authorities of these institutions. Also I am much indebted to Professor MORGAN, Doctor BRIDGES and Doctor STURTEVANT for valuable suggestions and advice.

ORIGIN OF CRIPPLED

The new mutant character, crippled, of *Drosophila melanogaster* was originally found in a black purple cinnabar fly. A female fly from the black purple cinnabar stock of the COLUMBIA laboratory was crossed with a male from the Swedish wild stock which had been kept breeding in the same laboratory. One of the F_1 wild-type females was backcrossed with a male of the former stock, and a wild-type female of the offspring was again backcrossed with one of her black purple cinnabar brothers. One black

purple cinnabar female from the backcross was found to have her left hind tarsus curved sharply forward. This female was the ancestor of all the "crippled" flies which were found in the subsequent experiments and showed abnormality in the hind or middle leg.

Later some flies with the same kind of abnormality were found in the original black purple cinnabar stock. These gave by breeding some crippled offspring which showed no difference in the genetical behavior from the other crippled flies, so that it is very likely that this new mutant had arisen in that stock some time before it was first found.

TWO TYPES OF CRIPPLED, "CRIP-H" AND "CRIP-M"

This mutant character is a rather remarkable one because of its high variability. The middle or hind leg becomes abnormal if the fly has this factor in the homozygous state. When the character appears in the hind leg, some of the segments of this leg are shortened, or broadened, or crooked, or twisted, etc., but when the middle leg is affected by the presence of apparently the same factor, it forks or dwindles, or entirely disappears. And evidently the state of the food of the larva determines whether the hind or middle leg is to become abnormal. In the following descriptions this character will be called, for the sake of convenience, "crip-h" or "crip-m" according as it appears in the hind or middle leg, and simply "crippled," when there is no need of discrimination.

CRIP-H

Description

Crip-h is a highly variable character. The fly homozygous for its factor may be quite normal somatically, but often has one of the hind legs more or less abnormal. Any of the segments of the leg may be shortened, broadened, straightened, crooked or twisted. Sometimes the abnormality is restricted to a single segment, but at other times more than one segment are deformed. Also it is quite frequent that the leg lacks some of the distal segments. In the extreme cases the entire leg is missing, leaving no trace at all. Figure 1 shows some of such crip-h legs.

Except in the cases where the abnormality is very slight, the crippled leg is apparently no longer functional; the fly drags it while walking. Moreover, such a fly often has one wing folded, owing to the hind leg of the same side being crippled and incapable of extending it.

As mentioned above, this kind of abnormality occurs as a rule in only one of the hind legs. But rarely both of the legs become abnormal at the same time; in these cases there is no similarity in the grade or type of the

abnormality of the two legs. Exceptionally the middle leg also shows the same kind of abnormality; in these cases the hind leg of the same side is also abnormal, practically without exception, and the abnormality is usually greater in degree in this leg than in the middle leg. Such cases of the abnormality of the middle leg belong to a category different from crip-m, as will be mentioned later.

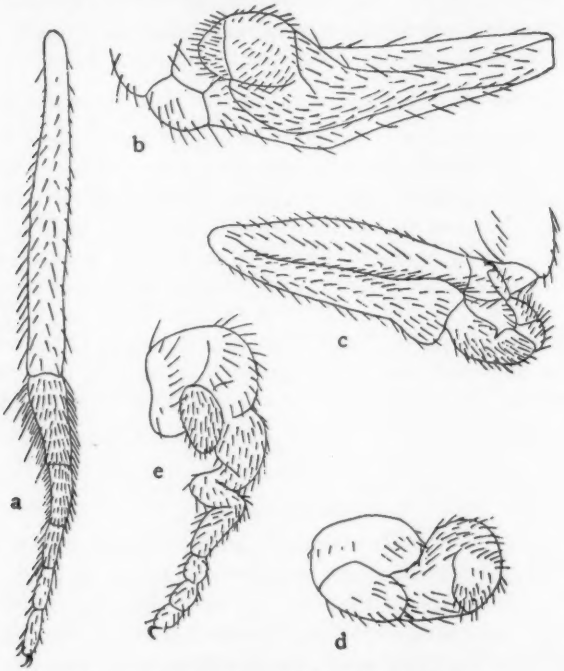

FIGURE 1.—Some forms exhibited by crip-h legs.

(a) Tibia straightened; first and second tarsal segments slightly broadened and curved. (b) Femur somewhat deformed; tibia swollen considerably at distal end; tarsal segments fused into spoon-shaped body, terminal claws missing. (c) Femur and tibia somewhat deformed; tarsus curved sharply forward and upward, with segments, especially the proximal two, swollen and deformed. (d) Femur crooked; tibia and tarsus missing. (e) Entire leg shaped something like a cork-screw; femur much shortened; tibia and proximal part of tarsus twisted. ×70.

Influence of environment

Age of parents

Before making the experiments on the effect of environment upon the character crip-h, special attention was given to whether the age of parents

had any effect on that character in the offspring. Thus, a pair of flies homozygous for the factor were left to lay eggs in the first bottle for eight days, and before the flies of the next generation began to emerge, they were transferred to a second bottle and left for the next eight days. Sometimes a third bottle was prepared in the same way. These two (or three) bottles were kept in the same incubator and the flies that emerged from them were counted and the percentages of the crip-h flies of the different bottles were compared. In all a dozen sets of bottles were prepared. Thus, I found that in some cases a relatively greater number of crip-h flies came out of the bottles of the later broods than from those of the earlier, but in other cases the reverse was true, and in still other cases the result was self-contradictory, that is, in one class there was a greater number of abnormal flies among the earlier broods than among the later, whereas in another class more crip-h flies were found among the later broods than among the earlier (classification being made in reference to some characters other than crip-h, for example, black, purple etc.). In short, the age of parents did not seem to play any definite rôle on the appearance of the crip-h character in the offspring.

High temperature

Of several pairs of bottles, prepared in the same way as described above, the first bottles were put into an incubator at a temperature 29° to 30° C, right after the removal of the parents, while the second bottles were kept in the 25°C incubator throughout. The percentages of the crip-h flies in the two types of bottles were compared. Sometimes the second brood was reared in the high-temperature incubator, while the first one was left in the 25°C incubator. The high temperature shortens the duration of the larval and pupal period more or less, sometimes by two days, so that the counting of the flies was given up on the eighth day after the first fly had appeared in the bottle. Thus, I found that, in seven out of eight sets of bottles there were relatively more crip-h flies in the high-temperature bottles than in the 25°C bottles, the difference amounting to 35 to 80 percent of the percentage of the crip-h flies in the 25°C bottle in most of the sets. In only one set the 25°C bottle yielded more crip-h flies than the corresponding high-temperature bottle. But in this set the brood of the former bottle was much smaller than that of the latter, and, as will be shown later, the percentage of the crip-h flies depends much on the size of the brood, the percentage being smaller the larger the brood, so that this case cannot be taken as contradictory to the general result.

Low temperature

A similar experiment was carried out for low temperature. The ordinary ice-box was used to get low temperature, which fluctuated from 8°C to 15°C. Such temperatures retarded the development of the larvae considerably, and the F_1 flies were counted for about a month after the first one had appeared. Most of the cultures produced less than one hundred flies during this time.

Of each set, consisting of the two bottles containing two successive broods, usually the first one, but sometimes the second one, was put in the ice-box. In all, ten sets of bottles were prepared.

The bottles kept in the ice-box produced relatively much larger numbers of crip-h flies than the corresponding bottles that had been kept in the 25° C incubator. The percentage of crip-h flies in the former was in most cases 65 to 75, whereas in the latter it was usually 20 to 30. It is clear then that, even if due allowance is made for the difference in the size of the broods of the cold and ordinary bottles, the difference in the percentages of crip-h flies was fairly great.

Three F_2 cultures from flies heterozygous for the factor for crippled were reared in the same ice box. From these cultures there appeared 17.7 to 24 percent crip-h flies, in all of which the abnormality was rather slight. A brood of black jaunty flies having no factor for crip-h, reared in the same ice-box, produced no crip-h flies among the progeny.

State of food

There is a more or less remarkable difference in the percentage of crip-h flies in the earlier and later counts of one and the same bottle. This difference is as a rule the more apparent the larger the brood. When the brood consists of as many as 300 to 600 flies, the percentage of crip-h flies in the last five days is often less than half that for the first five days. When the brood is smaller, this difference is less marked, and the percentage of crip-h flies in the bottle, taken as a whole, is higher than that of the other bottles containing larger broods, In the cultures in which more than one pair of crip-h flies were allowed to lay eggs, or in which the food was less than usual, this difference was especially marked. These facts show that this change in the percentage of the crip-h flies was closely associated with the change in the state of food induced by the larvae feeding on it. But it is not clear just what kind of chemical or physical change in the food is responsible for this change in the flies. This question will be taken up again later.

Inheritance

When a crip-h fly is crossed with a fly of an unrelated stock, most of the F_1 offspring are normal and very few are abnormal. The percentage of the abnormal flies is almost always lower than 1, sometimes being 0; in other words, this character shows recessiveness in the majority of cases, but a slight dominance in some exceptional cases. In F_2 appear numerous abnormal flies which are homozygous for the crip-h factor. The number of abnormal flies, however, is variable and always smaller than expected on the basis of a simple recessive factor, since flies that should be crippled, genetically, may be quite normal somatically, and one can tell only by breeding tests that they are homozygous for that factor.

The cross between the two flies homozygous for the crip-h factor gives usually 10 to 30 percent crip-h flies, more rarely 30 to 40 percent, the percentage practically never amounting to 50.

Evidently no special type of the abnormality is inherited. Not only do the crip-h flies of various types emerge from one and the same bottle, but a somatically normal pair of parents may produce a number of very abnormal flies, just as do somatically very abnormal pairs. Also, it seems to be determined at random which (right or left) of the hind legs is to become abnormal.

Locus of the factor for crip-h

Some black purple cinnabar crip-h males and females were crossed with star dichaete flies, and several of the F_1 star dichaete males were backcrossed to black purple cinnabar crip-h females. None of the crip-h flies that appeared in the next generation was star, except a few star crip-h flies which were tested, and found heterozygous for the crip-h factor. It is clear, then, that the factor for crip-h lies in the second chromosome. This fact was also proved by mating the black purple cinnabar crip-h fly with the wild fly and then backcrossing the F_1 fly with the black purple cinnabar crip-h fly. When the F_1 male was backcrossed with the black purple cinnabar crip-h female, all the crip-h flies that appeared in the next generation were also black purple cinnabar. When, on the other hand, the F_1 female was backcrossed, most of the crip-h flies of the next generation were also black purple cinnabar, but there appeared a few flies that were purple cinnabar (not-black) and crip-h.

The fact that the purple cinnabar (not-black) fly may be crippled, suggests that the factor for crip-h is located to the "right" of black. Accordingly, ten black (not-purple not-cinnabar) flies and sixteen purple cinnabar (not-black) flies which had come from crossing over between the

loci, black and purple, were tested for the crip-h factor. It was found that black (not-purple not-cinnabar) flies were never homozygous for the factor in question, while purple cinnabar (not-black) flies were homozygous for it without exception. These experiments have shown that the factor for crip-h probably does not lie between the loci black and purple, but is located between the loci purple and cinnabar. Next, six black purple (not-cinnabar) flies and seven cinnabar (not-black purple) flies which had come from the crossing over between the loci purple and cinnabar were tested. Five of the six black purple flies and five of the seven cinnabar flies were found to be homozygous for the crip-h factor, proving that the factor lies between the loci purple and cinnabar, which are known to be at about 54.5 and 57.5, respectively (BRIDGES and MORGAN 1919; CLAUSEN 1924; and MORGAN, BRIDGES and STURTEVANT 1925).

CRIP-M

Origin and description

In the course of the experiments described above, a somewhat different kind of crippled fly began to appear in some of the cultures. Such flies were first found in a certain strain, but appeared later also in a few other strains somewhat remotely related to it, and also to one another. In this particular kind of crippled the leg that becomes abnormal is one of the middle pair instead of the hind legs. Moreover, the abnormality belongs to a category different from the abnormality of the hind leg. The case most commonly met with has the leg entirely missing. More rarely it is represented only the coxa, or by the coxa and trochanter with or without a small vestige of some of the more distal segments (figure 2, g). Sometimes the leg is smaller in size than the normal leg, but is quite normal in shape and in proportional size of segments. In the majority of cases only one of the middle legs is abnormal, but rarely cases are found in which both of them are missing and the fly is four-legged.

Crip-m is a character sharply distinguishable from crip-h, and few cases have been met with in which the discrimination is hard. As mentioned above, crip-h flies may sometimes have the middle leg of the same side also somewhat abnormal. In these cases the middle leg is usually less abnormal than the hind leg. In crip-m, on the other hand, only the middle leg becomes abnormal, the hind leg remaining quite normal. Moreover, these two types of the crippled fly are distinguishable from each other by the time of appearance; crip-m flies appear only after the culture has become old, mostly during the last few days of counting when relatively few crip-h flies are appearing.

The number of the crip-m flies that appear from one culture is very small; it very rarely amounts to 5 to 8 percent of the total number of flies of the bottle.

Doubling of the middle leg

In crip-m flies the pleura of the thorax also often shows abnormality in some way or other. In the flies that lack one of the middle legs a depression is usually found at the part of the thorax where the leg should be. This depression may be so large as to cover the base of the sternopleural bristles, which are then also missing. In the flies with the middle leg smaller than normal, on the other hand, the pleura often has a sign of doubling as indicated by another set of the sternopleural bristles situated above the ordinary set. In such flies the part between the two sets of bristles commonly carries a rudiment of the leg. This rudiment is, as a rule, very small and imperfect, consisting of only one or a few segments (figure 2, a), but rarely it is fairly large and quite normal in proportion. There are even cases in which two practically perfect middle legs occur on the same side of the body (figure 2, b). There are also cases in which not the whole leg, but a part of it, is reduplicated, as shown in figure 2, c to f.

Such cases of the doubling of the leg remind one of the character "reduplicated" described by Miss HOGE (1915). "Reduplicated," however, was a sex-linked character and appeared in any leg, but predominantly in the fore leg, whereas the character before us is not sex-linked and appears exclusively in the middle leg. Moreover, the doubling seen here belongs to a category different not only from "reduplicated" but also from most of the cases of reduplication of the leg described in insects before. This fact may be clear from the following "rules" which have been formulated from observations on more than thirty cases of the doubling of the leg.

Rules of doubling

(1) The doubling may occur at any level of the leg.

(2) The parts contained in the branch are only those that are distal to the point of division.

(3) In all cases of doubling, one branch or one leg is in the normal position and direction.

(4) The plane of division of the leg into two primary branches may coincide with the vertical (dorso-ventral) plane or with the horizontal (antero-posterior) plane of the leg.

(5) When the division is by the vertical plane, the resulting two branches are mirror images of each other, the anterior surfaces facing.

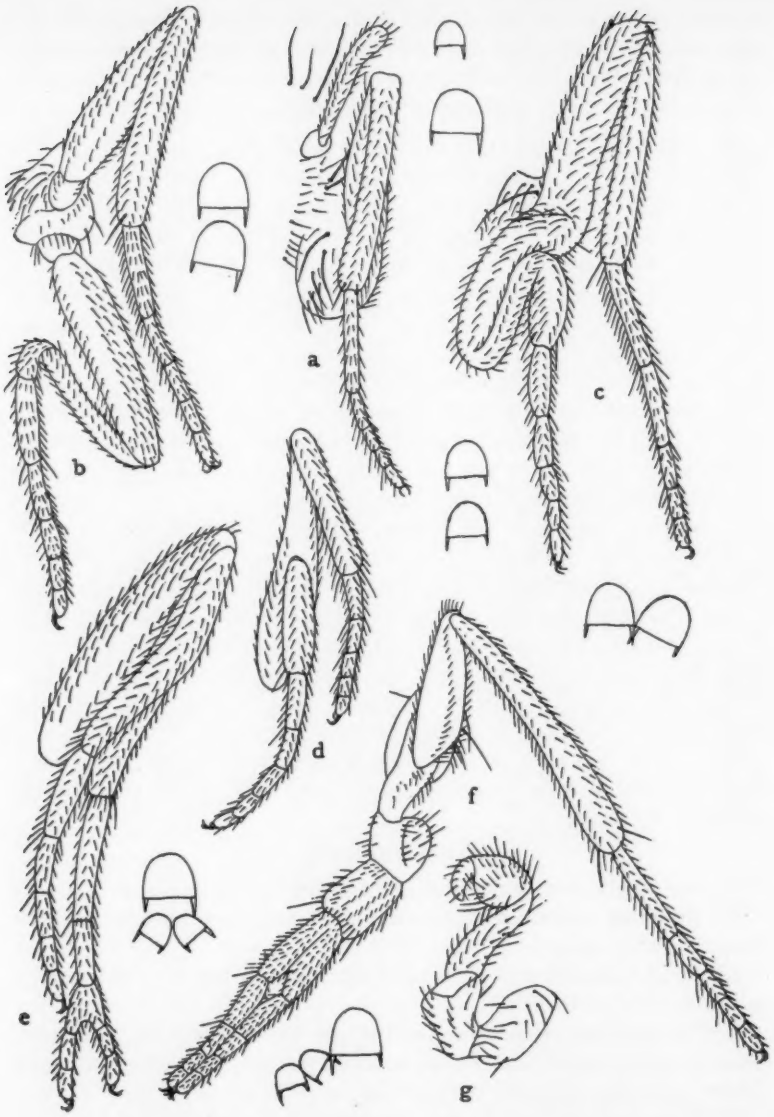
a
b
c
d
e
f
g

FIGURE 2. Median legs of crip-m flies. The accompanying diagrams show the side and direction of branches. All ×65.

(a) A small rudimentary median leg.

(b) Two nearly perfect median legs on the same side of the thorax.

(c) Left leg. Femur divided at base by a vèrtical (dorsi-ventral) plane; both branches complete, of about normal size, facing each other with anterior surface; supernumerary branch curved sharply near middle of tibia and folded up.

(d) Right leg. Limb, as large as the original limb and consisting of tibial and tarsal segments, attached on the ventral surface of the femur at about the middle of it; both limbs being right limbs.

(e) Left leg. Femur compound, that is, consisting of two branches fused into one, much thicker than normal; tibia bifurcated at base by horizontal (antero-posterior) plane, forming two branches, one overhanging the other; dorsal branch with tibia curved slightly; ventral branch with tibia, first and second tarsal segment compound, third divided by dorsi-ventral plane.

(f) Right leg. Trochanter divided by vertical plane; posterior branch normal in position and in proportion of parts; anterior branch with femur much shortened and deformed, tibia short and thick, compound; tarsus divided at first segment into two secondary branches with dorsal surfaces facing together.

(6) When the division is by the horizontal plane, the resulting two branches are of the same side (not mirror images of each other) and one hangs over the other.

(7) Of the two primary branches, the one in the normal position never undergoes further division, while the other may do so.

(8) When the secondary division occurs in the primary branch, the plane of division is usually vertical to the plane of the primary divisions,—if the primary plane is dorso-ventral, the secondary plane is horizontal, and *vice versa*.

(9) The two secondary branches are mirror images of each other.

The division of the leg by the horizontal plane into two branches, one overhanging the other, is very characteristic of the present case. As far as I know, few instances of this category have ever been described (Bateson, 1894, 1913). It is not uncommon here; in fact, I have observed more than a dozen cases belonging to this category.

Influence of state of food on crip-m

The crip-m flies appear almost without exception during the latter half of the counting, that is, from about the fifth day after the appearance of the first fly in the culture. This time coincides with the time when the percentage of the crip-h flies begins to lower. In a culture that throws relatively many crip-m flies the percentage of crip-h flies is comparatively low from the beginning. And if one keeps such a culture rather long, —more than one month—one commonly finds that the crippled flies that appear from it are mostly crip-m. It is thus very likely that one and the same cause is acting here both for the decrease of the percentage of the crip-h flies and for the production of the crip-m flies. And this cause is evidently environmental and connected with the change in the nature of the food, as shown by the results of the following two experiments:

First, two successive broods of crip-m flies were raised from the same parents; in all six such sets were prepared. In every set the two broods showed practically the same change in the type of the crippled flies from crip-h to crip-m.

In the second experiment, a pair of black purple cinnabar crip-m flies were introduced into a bottle, from which only black purple flies had been appearing for more than ten days and in which most of the crippled flies were then crip-m. Two out of ten such bottles produced offspring from the introduced pairs. The crippled flies among those offspring were mostly crip-m besides being black purple cinnabar.

The banana culture-medium is strongly acid at the start of the culture, then decreases its acidity gradually as many flies come out, and becomes weakly alkaline towards the end of the counting. I thought it might be this change in the chemical nature of the food that is mainly responsible for the change in the type of the abnormality of the fly. I therefore neutralized to a certain extent some new acid cultures with sodium bicarbonate or with ammonia water and also acidified some old alkaline cultures with acetic acid, but there was no recongizable effect on the percentage of the crip-h or crip-m flies that came out subsequently in either experiment. Some cultures were also kept extremely moist from beginning to end by adding water from time to time, but with no apparent effect. Thus, although it is likely that the change in question is induced by some change in the nature of the food, yet my experiments have failed to reveal just what kind of chemical or physical change is responsible for it.

Inheritance of crip-m

When crip-m is crossed with flies of an unrelated strain, the F_1 flies are all normal. When two crip-m flies are mated, there appear a number of crip-h offspring during the first half of the counting; then the number of the crip-h flies decreases; and, toward the end of the counting, a few crip-m flies come out. The result is just the same when two crip-h flies of the same strain are crossed together or a crip-m fly is mated with a crip-h fly.

To find the locus of the factor for crip-m in the chromosome, a few black purple cinnabar crip-m flies were mated with wild flies, and four F_1 females were backcrossed with black purple cinnabar crip-m males. From each bottle there appeared a few crip-m flies besides a number of crip-h flies. All of the crip-m flies were black purple cinnabar, except one black purple (not-cinnabar) and one cinnabar (not-black not-purple) crip-m fly. This result shows that the factor for crip-m character lies in the second chromosome somewhere between purple and cinnabar. Next, five crip-m females were mated, each with a male from the original black purple cinnabar stock bottle. As mentioned before, the flies from this stock may have carried the factor crip-h, but there is no reason to suspect that the factor for crip-m, if any special factor for this character exists, has been present in them. These five females all gave a few crip-m offspring besides a number of crip-h flies, showing that the factor for crip-m is probably the same as that which produces crip-h.

It is not known why no crip-m flies had been found at the beginning of this whole experiment on cripplied, but appeared later in some particular cultures. Possibly we have to deal with a modifying factor here. However, the number of crip-m flies that appear in each culture is too small to enable us to decide this question easily.

It might be suspected that the absence of the middle leg and the reduplication of the same would possibly represent entirely different characters. But this is evidently not the case. In fact, I often mated two flies having a reduplicated middle leg together, and also such a fly with a fly lacking a middle leg; these crosses always gave practically the same results as the cross between two flies lacking the middle leg. Moreover, there were series of types of abnormality of the middle leg from the doubling of the leg to its complete absence.

SUMMARY

1. Crippled is a new mutant character which appears in one of the middle or hind legs of *Drosophila melanogaster*. When it appears in the hind leg (crip-h), some segment or segments of this leg are shortened, or broadened, or crooked, or twisted or entirely missing. When it appears in the middle leg (crip-m), this leg forks, or dwindles, or entirely disappears.

2. Crippled is a recessive character. The cross between two flies homozygous for the factor gives usually 10 to 30 percent crippled offspring, most of which are crip-h and a few may be crip-m.

3. The percentage of the crip-h flies is higher in broods reared in high temperature (29° to 30°C) or low temperature (8° to 15°C) than in those bred in the optimum temperature (25° C).

4. The percentage of the crip-h flies that appear from the same bottle is higher during the first five days than during the last five days of the counting.

5. The factor for crip-h lies in the second chromosome between the loci purple and cinnabar.

6. The crip-m flies appear only near the end of the counting when the percentage of the crip-h flies is comparatively low.

7. The decrease of the percentage of the crip-h flies and the production of crip-m flies near the end of the counting, seem to be due to one and the same cause, namely, a certain state of the culture medium; but several attempts to identify the cause were unsuccessful.

8. When the reduplication of the middle leg occurs, the leg may be divided either by the vertical or by the horizontal plane. When it is divided by the vertical plane, the resulting branches are mirror images

of each other; when it is divided by the horizontal plane, the branches are of the same side, not mirror images.

9. One and the same factor probably produces both crip-h and crip-m.

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ON THE OCCURRENCE OF MUTATIONS WITHIN TRANSPLANTABLE NEOPLASMS[1]

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INTRODUCTION

In a survey of the recent work on the inheritance of susceptibility to a transplantable tumor, two general conclusions of importance can be deduced. The first of these conclusions formulated by LITTLE and STRONG (1924) is that the fate of the implanted tumor tissue is determined by a reaction between the host and the transplantable tumor cells. The reaction of the host is governed largely by its genetic constitution (derived from the zygote from which it arose) and that of the transplantable tumor is somewhat controlled by certain intrinsic or genetic factors. The expression "reaction potential" perhaps defines this play of factors which determine the "transplantability" of a tumor more accurately than the older expressions "proliferative or growth vigor" and "adaptability." These older expressions overemphasize the rôle of the tumor transplant at the expense of the important part played by the host.

The second conclusion, concerning the host's tolerance of a transplantable tissue is more specific, "susceptibility to a transplantable neoplastic tissue is brought about by the simultaneous presence of *multiple* mendelian (genetic) factors, introduced into the individual at the time of the fertilization of the egg" (LITTLE and STRONG 1924). With the use of the term "simultaneous presence," we do not intend to imply any reference to the discarded theory of presence and absence first introduced by Bateson. We use the term "presence" for purely descriptive purposes.

[1] The experiment here reported has been made possible by a grant from the Rockefeller Institute for Medical Research.

For example, if susceptibility to a transplantable tumor depended upon the simultaneous presence of two genetic factors, *A* and *B*, (as in the case of the adenocarcinoma, *dBrB*), then the mice with the following genetic constitutions would be susceptible; *AABB*, *AaBB*, *AABb* and *AaBb*. The mice with the following genetic constitutions would be resistant to the transplant; *AAbb*, *Aabb*, *aaBb* *aaBB* and *aabb*.

The Mendelian expectations for susceptible and non-susceptible individuals, involving the several possible combinations of "simultaneous factors," for the two generations, the F_2 and the backcross to the negative strain, are given in table 1.

TABLE 1

Expected proportions of susceptible (+) and non-susceptible (−) individuals in the two important generations, the F_2 and the backcross to the negative strain, on the assumption that for the continued growth of the transplant there must be present one or more definite genetic factors within the host.

NUMBER OF FACTORS	FACTORIAL COMPOSITION OF F_1	EXPECTATIONS F_2	RATIO IN F_2	PERCENT NEGATIVES F_2	EXPECTATIONS, BACKCROSS
1	*Aa*	3+ : 1−	1+ : 0.33−	25.00	1+ : 1−
2	*AaBb*	9+ : 7−	1+ : 0.78−	43.75	1+ : 3−
3	*AaBbCc*	27+ : 37−	1+ : 1.37−	57.81	1+ : 7−
4	*AaBbCcDd*	81+ : 175−	1+ : 2.16−	68.38	1+ : 15−
5	*AaBbCcDdEe*	243+ : 781−	1+ : 3.21−	76.27	1+ : 31−
6	*AaBbCcDdEeFf*	729+ : 3367−	1+ : 4.61−	82.20	1+ : 63−
7	*AaBbCcDdEeFfGg*	2187+ : 14197−	1+ : 6.49−	86.65	1+ : 127−
8	*AaBbCcDdEeFfGgHh*	6561+ : 65536−	1+ : 9.98−	90.89	1+ : 255−

EXPERIMENTAL METHODS

The tumor which is the subject of this investigation is the third spontaneous neoplasm to arise in a relatively homogeneous dilute brown strain of mice which had been rigidly inbred, brother to sister, for at least forty generations. This tumor, which has not been previously described, we have designated as *dBrC*, in order to continue the nomenclature used for the other tumors arising in this same stock which were called *dBrA* and *dBrB*. On transplantation it was found, as with the previous tumors, that all animals of this dilute brown strain were uniformly susceptible. On the other hand, the BAGG albino strain, another inbred stock, was

found to be uniformly resistant to transplants of this tumor as it had been to tumors A and B of the same stock.

The F_1 generation produced by crossing these two stocks gave individuals all of which grew the transplant (180+ : 0−). At the present time, 125 individuals from the F_2 generation have been inoculated with this transplantable tumor, *dBrC*, and 23 of these grew the transplant, while 102 proved to be resistant. In attempting to fit the observed data to mendelian principles, as has already been so successfully done in the case of four transplantable tumors (two by Tyzzer and Little and two by Little and Strong 1924), it was evident that the behavior of the transplant placed it in the same class as the two other transplantable tumors derived from this same inbred dilute brown stock of mice. The results can be explained if the assumption be made that for the successful growth of the transplant there must be present within the host at least from five to seven independently inherited genetic units or factors (genes). It is probable that the number of genetic units involved is six. The degree of probability determined by the comparison of the probable errors between observation and expectation for five, six and seven factors, is greater in the case of six factors than it is for the other two possibilities, although the five and the seven factor interpretation is not definitely excluded by the present data.

The degree of significance between the observation on the original *dBrC* tumor and the expectation according to Mendelian principles is given in table 2.

TABLE 2.

Comparison of the observed data for the F_2 generation with the expectation for five, six and seven Mendelian factors, in case of original dBrC tumor.

	F_2 GENERATION	NUMBER OF INDIVIDUALS	PERCENT NEGATIVES	DIFFERENCE BETWEEN
1	Observation	23.00+ : 102− ±2.89	81.60±2.30	
2	Expectation 5 Factors	29.69+ : 95.31− ±3.17	76.09±2.69	1 and 2=5.51%±3.53 or 1.56×PE
3	Expectation 6 Factors	22.28+ : 102.72− ±2.85	82.16±2.30	1 and 3=0.56%±3.25 or 0.17×PE
4	Expectation 7 Factors	16.69+ : 108.31− ±2.54	86.65±2.03	1 and 4=5.05%±3.05 or 1.65×PE

After the collection of the above data, no further genetic studies being contemplated, the tumor was simply continued for some generations in the original dilute brown stock. In the course of this routine continuation of the tumor it was found that one of the transplants grew with remarkable rapidity. In sub-transplants from this particular tumor the rapidity of growth continued. Whereas the original *dBrC* tumor, growing in the susceptible homogeneous dilute brown mice, would rarely attain a weight of a gram and a half to two grams in from two to three months, this single mass has produced upon further transplantation growths which have reached from twenty-two to twenty-three grams in from four to five weeks. Obviously there must have been a clear cut variation from the type reaction which had been investigated for over a year. Consequently this new tumor was called *dBrCX*, the *X* being attached to the original formula signifying some unknown characteristic of the new transplantable tumor. On further study it soon became apparent that the substrain had developed new tissue characters. *dBrCX* grew in all F_2's, in all F_1's, and in all individuals belonging to the original dilute brown mice, in all individuals belonging to several backcross generations toward the original non-susceptible stock, in all individuals belonging to the original non-susceptible stock, and in fact in all mice irrespective of their genetic relationships. It grows equally well in wild mice and mice belonging to a stock of mice imported from England several years ago and kept entirely distinct by brother-to-sister matings since their importation. From a state of high specificity, the transplantable tumor had become completely non-specific. Correlated with this loss of tissue specificity there was also obtained an increased growth or proliferative vigor. There was apparent a degree of significance of difference between the original *dBrC* and the new *dBrCX* tumor amounting to 32.57 times the probable error. From the time at which this distinct deviation from the normal growth rate of the transplantable tissue was observed we made careful observations with the possibility of detecting other deviations from the estimated normal growth rate. As a result of this procedure, two transplants were selected which seemed to show a difference. These have since received the names of *dBrCm* and *dBrCsp*. *dBrCm* will grow in a given host, provided there be apparent the simultaneous functioning of at least two independently segregating Mendelian units, that is, there is obtained a typical 9 : 7 ratio in the F_2 generation. The ratio of susceptible mice to non-susceptible mice in the F_2 generation for the *dBrCsp* tumor is probably 3 : 1. In other words, the physiological difference between susceptibility and non-susceptibility is apparently controlled by the functioning of one

TABLE 3

The data obtained for the three transplantable tumors, dBrCm, dBrCsp and dBrCX, in the F_2 generation, together with the comparable data for the original dBrC tumor.

NAME OF TUMOR		F_2 GENERATION	NUMBER OF INDIVIDUALS	PERCENT NEGATIVES	DIFFERENCE BETWEEN
Original *dBrC*	1	Observation	23.00+ : 102.00− ±2.89	81.60% ±2.30	
dBrCm	5	Observation	99.00+ : 65.00− ±4.17	39.64% ±2.53	1 and 5=41.96% ±3.42 or 12.26×PE.
	6	Expectation 1 Factor	123.00+ : 41.00− ±3.55	25.00% ±2.16	5 and 6=14.64% ±3.32 or 4.41×PE.
	7	Expectation 2 Factors	92.25+ : 71.75− ±4.23	43.75% ±2.57	5 and 7= 4.11% ±3.60 or 1.14×PE.
	8	Expectation 3 Factors	69.20+ : 94.80− ±3.60	57.81% ±2.20	5 and 8=18.17% ±3.35 or 5.42×PE.
dBrCsp	9	Observation	75.00+ : 23.00− ±2.79	23.47% ±2.86	1 and 9=58.13% ±3.67 or 15.84×PE.
	10	Expectation 1 Factor	73.50+ : 24.50− ±2.85	25.00% ±2.90	9 and 10= 1.53% ±4.07 or 0.37×PE.
	11	Expectation 2 Factors	55.10+ : 42.90− ±3.27	43.75% ±3.36	9 and 11=20.28% ±4.41 or 4.59×PE.
dBrCX	12	Observation	243.00+ : 2.00− ±0.94	0.82% ±0.38	1 and 12=80.78% ±2.48 or 32.57×PE.

Mendelian dominant factor introduced into the individual with the formation of the zygote. The data obtained for the three transplantable tumors, *dBrCm*, *dBrCsp* and *dBrCX* in the F_2 generation together with the comparative data for the original *dBrC* tumor are given in table 3.

RESULTS

A transplantable tumor, known as *dBrC*, gave very uniform results in a series of mice of known pedigree for a period of about a year. We may say that the reaction potential existing between the tumor and the individuals inoculated was the same during this first part of the experiment. This tumor will grow in a mouse progressively provided there be present simultaneously at least from five to seven independently segregating genetic factors (genes) in the fundamental make-up of the host derived from the zygote that gave rise to the individual. In the course of routine transplantation of this tumor, there was a sudden change in this hitherto constant reaction potential resulting in at least three types of transplantable tumors—new types as far as their physiological activity and reaction potential are concerned. With this change in the reaction potential of the transplantable tumor there is also involved or correlated with it a changed proliferative vigor and other physiological changes. From a single original transplantable mass, I have therefore obtained four masses that remain true to type at least for some months of continued experimentation. These four types are (1) the original tumor, *dBrC*, still giving a 5–7 mendelian factor ratio, (2) *dBrCm* giving a two factor ratio, (3) *dBrCsp* showing a one factor ratio, and (4) *dBrCX* growing in all mice inoculated—that is non-specific. The number of factors involved in the "transplantability" for the several tumors investigated is given in table 4.

TABLE 4

Tabulated synopsis of the various transplantable tumors used in the present experiment, showing the probable number of independently segregating genetic factors involved in the process of transplantation.

NAME OF TUMOR	NUMBER OF GENETIC FACTORS
Original *dBrC*	6
dBrCm	2
dBrCsp	1
dBrCX	Undetermined.

GENERAL DISCUSSION

Transplants from a spontaneous cancer rarely grow well in the first generations. Generally, however, the number of successful takes increases with subsequent transplantation till a very high rate is attained. There are two important theories formulated to explain this supposed increased ease of transplantation. The first theory may be called the virulence theory, although this term is rather unfortunate on account of certain implications from the study of infectious diseases. The second theory has been called the adaptation theory and is described by Woglam (1919) as "The presence (or absence) of power to adapt themselves to new hosts appears to be a deciding factor in the success (or failure) of most spontaneous mouse carcinomata after transplantation." The first theory is fostered by Ehrlich and Apolant, the second by Bashford, Murray, Haaland, Bowen, Cramer and more recently by Woglom.

The data contained in this paper have a direct bearing upon these two theories. It is very probable that neither the theory of adaptation nor the theory of virulence will explain completely the evidence outlined. These theories are descriptive phrases applied to the tumor mass and enter into the equation after the change or changes have been produced—that is, they are the result of the change and not the cause of the phenomenon. As in the case of so many other alternative hypotheses, a third explanation is sometimes called for that seems to fit the data better than either of the original hypotheses.

Before attempting to formulate this new hypothesis, let us enumerate a few of the features involved in the transplantation of malignant tumors, that have a bearing on the problem at hand. (1) The cell is the unit of structure in the malignant tissue as well as it is in the normal tissue. (2) The inoculation of a given mass consisting of malignant tissue results in a true transplantation of tissue and is not an infectious process. By this is meant that the cells making up the mass of the new growth are lineal descendants from the cells introduced into the host at the time of inoculation. (3) Very few cells placed in the new host give rise to the new growth; most of them die during the process. (4) Uniformity of behavior of the transplantable tumor implies, of course, that there must be a relatively stable mechanism for the perpetuation of this uniformity.

With these conceptions concerning the process of the transplantation of malignant tissue before us, we are now ready to attempt an analysis of this phenomenon of the change in the reaction potential encountered in

the present experiment. The change, whatever it is, must of course be either in the host component or in a possible tumor variable.

Obviously, if the host's tolerance of a transplantable tumor is dependent to a large extent at least upon its genetic constitution, when a change occurs in the genetic constitution of the host then this change, of necessity, would alter the reaction potential under consideration. Changes in the genetic constitution of the mice, however, cannot explain the present data. The second filial generation individuals, which give the indication of the genetic complex involved so far as the host is concerned have been picked out at random and inoculated with one type of transplantable tumor or another. The parents were the same for all the F_2's, while the number of susceptible grandparents was very small. Sometimes a single mouse was inoculated with two different types of tumors simultaneously. The genetic constitution of the mouse, as far as this experiment on testing its physiological response to two distinct types of tumors is concerned could not possibly be different. For these two reasons, the host complex during this experiment must have been a constant one or, at least, has not appreciably influenced the observed change in the reaction potential. The variable factor has not been within the mice employed.

We are therefore led to the conclusion that the only other variable that may be functioning is the transplantable tissue. There are two alternative conceptions concerning the mechanism of how this change in the tumor may have been brought about. (1) The original transplantable tumor employed was a mixed tumor, or (2) There has been a change in the genetic constitution of the unit of structure of the transplantable mass.

For several very obvious reasons, that need not be entered into here, the original tumor could not have been a "mixed" tumor.

The other conception is that this change of the reaction potential existing between the host inoculated and the tumor is brought about by an internal change in the genetic constitution of the tumor cell. This conclusion of course implies that the tumor cell is controlled to some extent at least by an internal genetic constitution.

Changes within the genetic constitution of living forms, if they are germ cells are called mutations; if they are somatic cells (occurring especially in plant tissues) are called somatic mutations. The conclusion is therefore reached that somatic mutations may occur within the tumor cell, and that when these mutations do occur, they change the reaction potential and other physiological activities of the tumor cell. The data at hand are not sufficient to determine more in detail the nature of this mutational

process. It may be either a change or shifting of a complete chromosome or chromosomes, or a change or changes within a chromosome or chromosomes (genic), or it may be even cytoplasmic in nature. By mutation, I merely mean to use the term in its broadest sense, that is, a change or shift within the genetic or internal constitution that results in definitely clear cut or discernible differences in behavior or structure that is perpetuated by the process of heredity (in this case, cell division).

We have therefore in the analysis of the data obtained in this experiment an explanation of the phenomenon of the so-called adaptiveness or virulence of the tumor cell. I have already pointed out the similarity between the data outlined in this paper and the data upon which the theories of adaptiveness and virulence or proliferative vigor are founded. If a genetic change such as a somatic mutation is responsible for the change in the reaction potential of the transplantable tumor cell and other physiological activities, such as the growth vigor then it is highly probable that the real cause of the phenomenon has been determined. A tumor increases its proliferative vigor or manifests an increased adaptiveness as a result of this mutational process. This somatic mutation may occur within a single cell in the neoplastic tissue. Then by the process of selective vitality or adaptiveness of the cells in the mass, the new type of behavior is obtained.

One other point I desire to mention. If one assumes that the spontaneous tumor possesses the same genetic constitution as the host that gave rise to it (and we have every reason to suppose that it does have, since the tumor has arisen by some process or other from cells derived from the body of the normal individual) then we must conclude from our present data, that a tumor mass may deviate from the genetic constitution of the host from which it arose, at least during the process of transplantation.

SUMMARY

1. Mutations or hereditary genetic changes occur within the tumor mass at least during the process of transplantation.

2. These mutations result in a changed physiological activity of the tumor mass.

3. The theory of mutation explains the old phenomenon of the increased percent of successfully growing masses on continued transplantation that has until the present been explained by the theories of adaptation and virulence.

4. The nature of this mutational process is as yet undetermined.

5. The tumor mass may deviate from the genetic constitution of the host that gave rise to it, at least during the process of transplantation.

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